

RESPONSE

I. Status of the Claims

Claims 1, 2 and 6-9 are presently pending in this case.

II. Rejection of Claims Under 35 U.S.C. § 101 and 35 U.S.C. § 112, first paragraph

The Action rejects claims 1-2 and 6-9 under 35 U.S.C. § 101 and § 112, allegedly because they are drawn to an invention with no apparent or disclosed patentable utility.

While in no way agreeing with the Examiner's remarks in the present Action, Applicants current response will summarize and concentrate on the overwhelming evidence, previously provided in Applicant's many Response to Office Actions (dated July, 1, 2002; December 26, 2002 and April 6, 2004) and in both the Appeal Brief and RCE filed in this case (all of which are herein incorporated by reference in their entirety).

The instant Specification, as filed, asserted that the claimed sequences encode a novel human G-protein coupled receptor (NGPCR). It also discusses the utility and activity of GPCRs including describing the tissue expression pattern of this NGPCR (on page 5) and making disease associations "NGPCRs may be important targets for the therapeutic treatment of, *inter alia*, diabetes, abnormal body weight or obesity, atherosclerosis, heart disease, abnormal blood pressure, cancer, and any associated symptoms." (specification at page 4, lines 23-27).

In their multiple Responses, Applicants have provided additional evidence regarding the physiologic activity of this NGPCR by describing the results obtained when knockout mouse were made as was described at multiple locations within the specification (page 2, line 32 through page 3,

line 6; page 4, line 4; page 6, line 6; page 26, lines 7-15). In these mice, the murine ortholog of SEQ ID NOS: 1 and 2 of the present invention was disrupted by homologous recombination (as described in the specification) and the homozygous (-/-) knockout mice, heterozygous (+/-) mice and wild-type (+/+) were subject to a medical work-up using an integrated suite of medical diagnostic procedures designed to assess the function of the major organ systems in a mammalian subject. Disruption of the mouse homologue of the gene defined by the sequences of the present invention and thus elimination of the protein it encodes, resulted in neonatal lethality with 3 of the 9 homozygous (-/-) knockout mice born dying at approximately 1 day after birth. The remaining 6 homozygous(-/-)knockout mice exhibited muscle and limb control problems and died at 3 to 4 weeks of age. Heterozygous (+/-) male mice exhibited slightly increased mean systolic blood pressures when compared with their gender-matched wild-type (+/+) littermates and the historical mean. Systolic blood pressure was measured via a noninvasive tail-cuff method for four days on the Visitech BP-2000 Blood Pressure Analysis System. The blood pressure was measured ten times each day for the four days and then averaged to obtain a mouse's conscious systolic blood pressure. This provides clear evidence that the nucleic acid and protein encoded by the sequences of the present invention have a critical biological role and any disruption of the function of this protein results in alterations in blood pressure and even death. Clearly, the sequences of the present invention, the molecules which they encode and agonists or antagonists directed at them, can be used, as provided in the specification as filed, to diagnose and treat vascular disorders such as, *inter alia*, atherosclerosis, heart disease, and specifically abnormal blood pressure (specification at page 4, lines 6-10).

Furthermore Applicants have provided evidence that a sequence sharing greater than 93% identity at the amino acid level with the sequences of the present invention was present in the leading scientific repository for biological sequence data (GenBank), and has been annotated by third party scientists *wholly unaffiliated with Applicants* as *Homo sapiens* putative vascular inducible G protein-coupled receptor (VIGR) mRNA (GenBank accession number AAO13250, alignment and information provided in previous responses).

In spite of the previously submitted evidence that Applicants assertions of utility, as made in the specification as filed were credible, the Examiner has chosen to assert that the present case is most akin to Example 12, wherein the utility of a GPCR is not credible and again attempts to use contrarian art, much of which has been previously addressed and discredited by Applicants in the previous responses described above and which for brevity's sake will not be repeated here .

Applicants herein submit that the fact pattern of the present case is not like Example 12 (Action at page 5, line 19) but rather more like that of Example 10. Therefore, the pending rejection of the present invention due to lack of patentable utility runs contrary to Example 10 of the PTO's Revised Interim Utility Guidelines Training Materials (pages 53-55), which establishes that a rejection under 35 U.S.C. § 101 as allegedly lacking a patentable utility and under 35 U.S.C. § 112, first paragraph as allegedly unusable by the skilled artisan due to the alleged lack of patentable utility, is not proper when there is no reason to doubt the asserted utility of a full length sequence that has a similarity score of 95% to a protein having a known function. The sequences of the present invention encode a protein that is known as vascular inducible G protein-coupled receptor (VIGR) whose function has been described in a publication entitled "VIGR- a novel

inducible adhesion family G-protein coupled receptor in endothelial cells.” (Stehlik, C., *et al.*, FEBS Lett. 569(1-3):149-55, 2004, abstract provided as **Exhibit A**) . This publication regarding VIGR is associated with the GENBANK nucleic acid sequence entry NM_198569, and is also known as *Homo sapiens* G protein-coupled receptor 126 (Entry provided as **Exhibit B**). Alignment of t SEQ ID NO:1 of the instant invention and NM_198569 (provided as **Exhibit C**) reveals 100% identity over the 3753 nucleic acids or SEQ ID NO:1. Thus, the nucleic acid sequence of SEQ ID NO:1 is completely contained within NM_198569 and SEQ ID NO:1 therefore represents a shorter variant of the published VIGR protein that contains the functional domains and would therefore be expected to have the functions asserted in the specification as filed and supported by the published evidence.

It is long established that after filing disclosures can be used to support and verify assertions made in the specification. *In re Brana* (34 USPQ2d 1436 (Fed. Cir. 1995); “*Brana*”) it states “(t)he Kluge declaration, though dated after applicants’ filing date, can be used to substantiate any doubts as to the asserted utility since this pertains to the accuracy of a statement already in the specification”. In addition in *In re Jolles* (628 F.2d 1322, 206 USPQ 885, (CCPA 1980); “*Jolles*”), the establishes that only the assertion of utility must be present in the specification as originally filed, and, further, that post-filing evidence can be relied upon to support the original assertion of utility. Thus in view of the holdings in both *Brana* and *Jolles*, it is clear that the evidence is proper and that clarly those of skill in the art would find Applicant’s assertions of utility to be credible and believable.

The Examiner attempts to discredit Applicants assertions that the claimed sequences

encode VIGR with a handful of contrarian publications, many by the same individual, typically used by Examiners in an attempt to discredit the value of structure-function analysis and bioinformatics. As Applicants detailed in their previous response, even these publications in fact see great value in bioinformatics. Applicants believe that, contrary to these few publications, bioinformatic information is valued by the vast majority of those of skill in the art, the scientific community and that the numerous and frequent publication of such information speaks to its value. Rather than overwhelm the USPTO's scanning division with even the abstracts of the more than 5,548 different scientific publications that result of a search of the NCBI-NLM-NIH public scientific database "PubMed" using the term "bioinformatics", Applicants respectfully invite the Examiner to simply repeat it. Further Applicants note that there is even a Journal entitled "Bioinformatics" and that clearly this demonstrates the value those of skill in the art place on bioinformatic information.

Additional evidence supporting Applicants' "beliefs" regarding the value of bioinformatics include the fact that many scientists, corporations and institutions elect to allocate significant proportions of their limited resources for access to private bioinformatic systems and databases. Thus, it would appear obvious that if those of skill in the art value the results of bioinformatic analysis and are willing to pay dearly for access to such information that such information must have well recognized utility.

Furthermore, clearly indicative that those of skill in the art accept the value of bioinformatic information, is the fact that multiple US patents have been issued by the USPTO regarding bioinformatic prediction and methods for doing the same (see for example, U.S. Patent Nos. 6,229,911, 6,567,540, 6,615,141, 6,631,331, 6,651,008, 6,677,114, copies of issued U.S.

Patents not provided pursuant to current United States Patent and Trademark Office policy).

Clearly, if as the Examiner alleges the provided examples indicate, that bioinformatic analysis is without merit, then such patents would not have issued. Clearly a method or system for doing something that those of skill in the art recognize is without merit would lack patentable utility and said patents would not have issued if such were the case. However, multiple such patents have issued and therefore bioinformatic analysis and findings as well as methods and systems for doing bioinformatic analysis have utility and must logically, therefore, have merit.

Besides, there is likely no one of skill in the art that would argue that there is no structure-function relationship, particularly when two molecules share 100% identity. Even USPTO policy recognizes that there is no reason to doubt the asserted utility of a full length sequence that has a similarity score of 95% to a protein having a known function.

Therefore, the evidence in the present case clearly supports Applicants' assertions that the sequences of the present invention encode a novel variant of a human GPCR (VIGR), a protein whose function is known and therefore has established utility that is recognized by those of skill in the art. The present case is identical to that presented in Example 10 of the Revised Interim Utility Guidelines Training Materials (pages 53-55). In the present case it is clear that the sequences of the present invention encode a novel variant of VIGR with greater than a 95% identity to a protein having a known function, as asserted in the specification. However, even if, *arguendo*, Applicants had failed to assert this utility, according to the guidelines "Note that if there is a well-established utility already associated with the claimed invention, the utility need not be asserted in the specification as filed...Thus the conclusion reached from this analysis is that a 35 U.S.C. § 101 and

a 35 U.S.C. § 112 first paragraph, utility rejection should not be made”(emphasis added). Thus, the present rejection of the presently claimed invention under a 35 U.S.C. § 101 and a 35 U.S.C. § 112 first paragraph utility rejection should not have been made and should be withdrawn.

The Action also rejects claims 2, 7 and 9 under 35 U.S.C. § 112 because the specification, while being enabling for an isolated nucleic acid molecule encoding SEQ ID NO: 2 (such as SEQ ID NO: 11) or an isolated host cell, does not reasonably provide enablement for every isolated nucleic acid molecule that hybridizes under stringent conditions to SEQ ID NO: 1 or a non-isolated host cell that comprises a transgenic primate or human in origin or could result from human genetic therapy. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The Action rejects Claim 2 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention, because the specification, while being enabling for an isolated nucleic acid molecule encoding SEQ ID NO: 2 (such as SEQ ID NO: 1) does not reasonably provide enablement for every isolated nucleic acid molecule that hybridizes under stringent conditions to SEQ ID NO: 1. Applicants respectfully traverse this rejection.

35 U.S.C. § 112, first paragraph, requires that the specification contain a written description of the invention. The Federal Circuit in *Vas-Cath Inc. v. Mahurkar* (19 USPQ2d 1111 (Fed. Cir. 1991); “*Vas-Cath*”) held that an “applicant must convey with reasonable clarity to

those skilled in the art that, as of the filing date sought, he or she was in possession *of the invention.*” *Vas-Cath*, at 1117, emphasis in original. However, it is important to note that the above finding uses the terms reasonable clarity to those skilled in the art. Further, the Federal Circuit in *In re Gosteli* (10 USPQ2d 1614 (Fed. Cir. 1989); “*Gosteli*”) held:

Although [the applicant] does not have to describe exactly the subject matter claimed, . . . the description must clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed.

Gosteli at 1618, emphasis added. Additionally, *Utter v. Hiraga* (6 USPQ2d 1709 (Fed. Cir. 1988); “*Utter*”), held “(a) specification may, within the meaning of 35 U.S.C. § 112 ¶1, contain a written description of a broadly claimed invention without describing all species that claim encompasses” (*Utter*, at 1714). Therefore, all Applicants must do to comply with 35 U.S.C. § 112, first paragraph, is to convey the invention with reasonable clarity to the skilled artisan.

Further, the Federal Circuit has held that an adequate description of a chemical genus “requires a precise definition, such as by structure, formula, chemical name or physical properties” sufficient to distinguish the genus from other materials. *Fiers v. Sugano*, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993; “*Fiers*”). *Fiers* goes on to hold that the “application satisfies the written description requirement since it sets forth the . . . nucleotide sequence” (*Fiers* at 1607). In other words, provision of a structure and formula - the nucleotide sequence - renders the application in compliance with 35 U.S.C. § 112, first paragraph.

More recently, the standard for complying with the written description requirement in claims

involving chemical materials has been explicitly set forth by the Federal Circuit:

In claims involving chemical materials, generic formulae usually indicate with specificity what the generic claims encompass. One skilled in the art can distinguish such a formula from others and can identify many of the species that the claims encompass. Accordingly, such a formula is normally an adequate description of the claimed genus. *Univ. of California v. Eli Lilly and Co.*, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997).

Thus, a claim describing a genus of nucleic acids by structure, formula, chemical name or physical properties sufficient to allow one of ordinary skill in the art to distinguish the genus from other materials meets the written description requirement of 35 U.S.C. § 112, first paragraph. As further elaborated by the Federal Circuit in *Univ. of California v. Eli Lilly and Co.*:

In claims to genetic material ... a generic statement such as ‘vertebrate insulin cDNA’ or ‘mammalian insulin cDNA’, without more, is not an adequate written description of the genus because it does not distinguish the claimed genus from others, except by function. It does not specifically define any of the genes that fall within its definition. It does not define any structural features commonly possessed by members of the genus that distinguish them from others. One skilled in the art cannot, as one can do with a fully described genus, visualize or recognize the identity of members of the genus. (Emphasis added).

Thus, as opposed to the situation set forth in *Univ. of California v. Eli Lilly and Co.* and *Fiers*, the nucleic acid sequences of the present invention are not distinguished on the basis of function, or a method of isolation, but in fact are distinguished by structural features - a chemical formula, *i.e.*, the *sequence itself*.

The Action (page 6-7) rejects Claim 2, because it allegedly does not reasonably provide enablement for every isolated nucleic acid molecule that hybridizes under stringent conditions to

SEQ ID NO: 2. However, Applicants respectfully submit that Claim 2 has two limitations, the first being that molecules which encode the amino acid sequence shown in SEQ ID NO: 2; and the second being hybridization to the nucleotide sequence of SEQ ID NO: 2 or the complement thereof. Applicants submit that the nucleic acid molecules identified by the intersection of both parts of Claim 2, those that encode the amino acid sequence shown in SEQ ID NO: 2; and hybridize to the nucleotide sequence of SEQ ID NO: 1 or the complement thereof, is a finite and well defined group, which those of skill in the art could easily identify and would know how to make and use. Therefore, Applicants respectfully request that the rejection of Claim 2 under 35 U.S.C. § 112, first paragraph, be withdrawn

The Action also persists in rejecting claims 7 and 9 under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for a host cell in culture comprising a polynucleotide with the sequence as set forth in SEQ ID NO: 1, does not reasonably provide enablement for *in vivo* transfection. Furthermore, the Action persists in rejecting claims 7 and 9 under 35 U.S.C. § 101, allegedly because the claimed invention is directed to non-statutory subject matter as the Examiner asserts that any claim that could read on *in vivo* transfection could also be construed to read on transgenic humans, which therefore invalidates the claim as possibly drawn to non-patentable subject matter. Applicants respectfully note that, as described in previous responses hundreds, perhaps thousands of claims to host cells have been issued by United States Patent and Trademark Office and as issued U.S. Patents are presumed to meet all of the requirements for patentability, including of course statutory subject matter, how is it possible that the interpretation of this claim has changed such that it is now interpreted to read on non-statutory transgenic humans?

Applicants find it hard to believe that one could successfully assert in court that a claim to a host cell in an issued patent reads on subject matter that is recognized as non-statutory.

Furthermore, in the instant specification it states (on page 24, line 3) specifically “non-human primates” when referring to transgenic animals, thus this phrase actively excludes humans (human primates) from consideration thus eliminating any such concern specifically and obviating this rejection.

The Action also rejects claims 7 and 9 under 35 U.S.C. § 112, first paragraph, because it alleges that the specification, while being enabling for an isolated host cell, does not reasonably provide enablement for a non-isolated host cell, as such could comprise a transgenic primate or human in origin or could result from human genetic therapy. The Examiner further alleges that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the claimed invention, a host cell, commensurate in scope with these claims. Applicants disagree and respectfully traverse.

Specifically the Action (at pages 11-12) alleges that the disclosure does not provide adequate written description or any working examples by which genetic engineering or gene therapy could be accomplished.

First, the Actions position that the absence of “any working examples” is not dispositive, nor particularly relevant, as to the question of enablement, for it has long been established that “there is no statutory requirement for the disclosure of a specific example” (*In re Gay*, 309 F.2d 769, 135 USPQ 311 (CCPA, 1962)). Thus, this argument alone cannot support an allegation that claims 7 and 9 are not enabled.

The present application is fully enabling for the claimed invention - a host cell. Many host cells are described in the specification as filed and host cells are very well known to those of skill in the art. Applicants also note that claims directed at a "host cell" have been allowed in hundreds of patents which contain no more disclosure than the present case (see for example U.S. Patent Nos. 6,531,309, 6,586,230, 6,777,221: **Exhibits D-F**, copies of issued U.S. Patents not provided pursuant to current United States Patent and Trademark Office policy). As issued U.S. Patents are presumed to meet all of the requirements for patentability, including enablement under 35 U.S.C. §112, first paragraph. Therefore, Applicants respectfully submit that the present claim directed at a host cell must also logically be enabled and meet the requirements of 35 U.S.C. § 112, first paragraph.

Furthermore, Applicants herein present evidence of the state of the art with regard to making transgenic animals, at or before the time at which the present application was filed, which indicate that in fact the specification as filed is enabling for the generation of non-human transgenic animals. Applicants respectfully point out that there are numerous examples of transgenic worms (nematodes), mice, rats, rabbits, guinea pigs, pigs, birds (chickens), goats and monkeys, years and sometimes decades prior to the filing date of the present application. However, rather than provide hundreds of citations of transgenic animals that are in the art prior to the filing date of the present application, Applicants respectfully point out that the first report of a transgenic nematode was in 1988 (Spieth *et al.*, *Dev. Biol.* **130**:285-293; copy of abstract provided in **Exhibit G**), the first report of a transgenic mouse was in 1980 (Gordon *et al.*, *Proc. Natl. Acad. Sci. USA* **77**:7380-7384; copy of manuscript provided in **Exhibit H**), the first report of a transgenic rat was in 1990

(Mullins *et al.*, *Nature* **344**:541-544; copy of abstract provided in **Exhibit I**), the first report of a transgenic rabbit was in 1985 (Hammer *et al.*, *Nature* **315**:680-683; copy of abstract provided in **Exhibit J**), a report of the production of human interleukin-2 in the milk of transgenic rabbits was published in 1990 (Bühler *et al.*, *Bio/Technology* **8**:140-143; copy of abstract provided in **Exhibit K**), the first reports of transgenic guinea pigs were in 2000 (Suzuki *et al.*, *Gene Ther.* **7**:1046-1054, and Yagi *et al.*, *JARO* **1**:315-325; copies of abstracts provided in **Exhibit L**), a report of the production of human growth hormone in the milk of transgenic guinea pigs was also published in 2000 (Hens *et al.*, *Biochim. Biophys. Acta* **1523**:161-171; copy of abstract provided in **Exhibit M**), the first report of a transgenic pig was in 1985 (see **Exhibit J**), a report of the production of a heterologous milk protein in the milk of transgenic pigs was published in 1991 (Wall *et al.*, *Proc. Natl. Acad. Sci. USA* **88**:1696-1700; copy of manuscript provided in **Exhibit N**), the first reports of transgenic chickens were in 1987 (Salter *et al.*, *Virology* **157**:236-240; copy of abstract provided in **Exhibit O**) and 1989 (Bosselman *et al.*, *J. Virol.* **63**:2680-2689; copy of abstract provided in **Exhibit P**), the first reports of transgenic goats were in 1991 (Ebert *et al.*, *Bio/Technology* **9**:835-838, and Denman *et al.*, *Bio/Technology* **9**:839-843; copies of abstracts provided in **Exhibit Q**), the first report of a transgenic sheep (another example of a transgenic mammal) was in 1988 (Simons *et al.*, *Bio/Technology* **6**:179-183; copy of abstract provided in **Exhibit R**), and a report of the production of human anti-hemophilic factor IX in the milk of transgenic sheep was published in 1989 (Clark *et al.*, *Bio/Technology* **7**:487-492; copy of abstract provided in **Exhibit S**). Given the hundreds of reports of transgenic animals, of which the reports listed above are only the first examples, there can be no doubt that the making of transgenic

animals is clearly enabled to those of skill in the art, which is all that is required to meet the enablement requirement under 35 U.S.C. § 112, first paragraph.

The Examiner further seems to believe that claims 7 and 9 are not enabled for transgenic animals because certain aspects of transgenic technology (expression levels, site-specific *versus* random integration) require some level of experimentation to perfect. However, Applicants respectfully point out that all that is required in order to satisfy the enablement requirement under 35 U.S.C. § 112, first paragraph, is making any transgenic animal, not the perfect transgenic animal. Any detectable level of expression of a transgene, for example SEQ ID NO:1, is all that is required, for it is well established that the enablement requirement is met if any use of the invention (or in this case, certain aspects of the invention) is provided (*In re Nelson*, 126 USPQ 242 (CCPA 1960); *Cross v. Iizuka*, 224 USPQ 739 (Fed. Cir. 1985)). “The enablement requirement is met if the description enables any mode of making and using the invention.” *Johns Hopkins Univ. v. CellPro, Inc.*, 47 USPQ2d 1705, 1719 (Fed. Cir. 1998), citing *Engel Indus., Inc. v. Lockformer Co.*, 20 USPQ2d 1300, 1304 (Fed. Cir. 1991). Furthermore, a specification “need describe the invention only in such detail as to enable a person skilled in the most relevant art to make and use it.” *In re Naquin*, 158 USPQ 317, 319 (CCPA 1968); emphasis added. Therefore, as the skilled artisan is clearly able to make a variety of different species of transgenic animals, claims directed at non-human transgenic animals are thus enabled and are supported by a specification that provides sufficient description to enable the skilled person to make and use the invention as claimed.

Therefore Applicants respectfully request the withdrawal of the present rejection of claims 7 and 9 as the instant specification, in light of the state of the art, clearly enables those of skill in the art

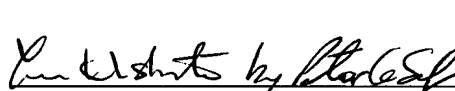
to make and use the claimed invention.

III. Conclusion

The present document is a full and complete response to the Action. In conclusion, Applicants submit that, in light of the foregoing remarks, the present case is in condition for allowance, and such favorable action is respectfully requested. Should Examiner Murphy have any questions or comments, or believe that certain amendments of the claims might serve to improve their clarity, a telephone call to the undersigned Applicants' representative is earnestly solicited.

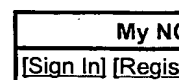
Respectfully submitted,

June 10, 2005
Date


Lance K. Ishimoto Reg. No. 41,866
Attorney for Applicants

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FULL-TEXT ARTICLE**VIGR--a novel inducible adhesion family G-protein coupled receptor in endothelial cells.****Stehlik C, Kroismayr R, Dorfleutner A, Binder BR, Lipp J.**

Department of Vascular Biology and Thrombosis Research, Medical University of Vienna, and BMT - Biomolecular Therapeutics GmbH, Brunnerstrasse 59, A-1235 Vienna, Austria. cstehlik@hsc.wvu.edu

Using a signal sequence trap for selection of differentially expressed secretory and membrane proteins, we identified a novel member of the adhesion family of G-protein coupled receptors (GPCRs), termed vascular inducible GPCR (VIGR). VIGR contains C1r-C1s, Uegf and Bmp1 (CUB) and pentraxin (PTX)-like modules and a mucin-like spacer, followed by seven transmembrane domains. By surface biotinylation as well as by immunofluorescence analysis we demonstrate that endogenous, highly glycosylated VIGR is expressed on the cell surface of endothelial cells (ECs) upon LPS or thrombin treatment, and inducible expression is mediated by MAP kinases, but not NF-kappaB. We show that VIGR is selectively expressed in ECs derived from larger vessels, but not from microvessels. In summary, VIGR represents a novel GPCR of the adhesion family, which is unique in its long extra-cellular domain comprising CUB and PTX-like modules and in its inducibility by LPS and thrombin in a subset of ECs, suggesting an important function in cell-adhesion and potentially links inflammation and coagulation.

PMID: 15225624 [PubMed - indexed for MEDLINE]

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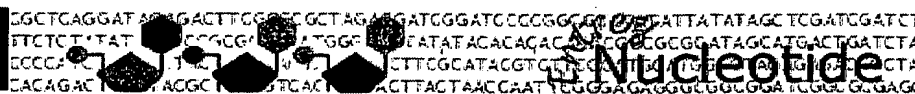
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LOCUS NM_198569 6902 bp mRNA linear PRI 23-APR-2005
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 VERSION NM_198569.1 GI:50355940
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 SOURCE Homo sapiens (human)
 ORGANISM Homo sapiens
 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
 Mammalia; Eutheria; Euarchontoglires; Primates; Catarrhini;
 Hominidae; Homo.
 REFERENCE 1 (bases 1 to 6902)
 AUTHORS Stehlik,C., Kroismayr,R., Dorfleutner,A., Binder,B.R. and Lipp,J.
 TITLE VIGR--a novel inducible adhesion family G-protein coupled receptor
 in endothelial cells
 JOURNAL FEBS Lett. 569 (1-3), 149-155 (2004)
 PUBMED [15225624](#)
 REMARK GeneRIF: VIGR represents a novel G protein coupled receptors of the
 adhesion family, which is unique in its long extra-cellular domain.
 REFERENCE 2
 AUTHORS Moriguchi,T., Haraguchi,K., Ueda,N., Okada,M., Furuya,T. and
 Akiyama,T.
 TITLE DREG, a developmentally regulated G protein-coupled receptor
 containing two conserved proteolytic cleavage sites
 JOURNAL Genes Cells 9 (6), 549-560 (2004)
 PUBMED [15189448](#)
 REFERENCE 3 (bases 1 to 6902)
 AUTHORS Fredriksson,R., Gloriam,D.E., Hoglund,P.J., Lagerstrom,M.C. and
 Schioth,H.B.
 TITLE There exist at least 30 human G-protein-coupled receptors with long
 Ser/Thr-rich N-termini
 JOURNAL Biochem. Biophys. Res. Commun. 301 (3), 725-734 (2003)
 PUBMED [12565841](#)
 REFERENCE 4 (bases 1 to 6902)
 AUTHORS Matoba,R., Okubo,K., Hori,N., Fukushima,A. and Matsubara,K.
 TITLE The addition of 5'-coding information to a 3'-directed cDNA library
 improves analysis of gene expression
 JOURNAL Gene 146 (2), 199-207 (1994)
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Feb 9 2005 14:31:10

FASTA searches a protein or DNA sequence data bank
version 3.3t05 March 30, 2000

Please cite:

W.R. Pearson & D.J. Lipman PNAS (1988) 85:2444-2448

/tmp/fastaCAA.jaOth: 3753 nt

>LEX41

vs /tmp/fastaDAA.jaOth library

searching /tmp/fastaDAA.jaOth library

6902 residues in 1 sequences

FASTA (3.34 January 2000) function [optimized, +5/-4 matrix (5:-4)] ktup: 6

join: 91, opt: 76, gap-pen: -16/ -4, width: 16

Scan time: 0.166

The best scores are:

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100.000% identity in 3753 nt overlap (1-3753:402-4154)

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	590	600	610
	220	230	240
LEX41	CTCCGAGCCCCACCGGTTATATCATTTCAGATAACATTTAACGACTTTGACATTGAAGAA		
gi 503	CTCCGAGCCCCACCGGTTATATCATTTCAGATAACATTTAACGACTTTGACATTGAAGAA		
	620	630	640
	650	660	670
	280	290	300
LEX41	GCTCCCAATTGCATTTATGACTCATTATCCCTTGATAATGGAGAGAGCCAGACTAAATTT		
gi 503	GCTCCCAATTGCATTTATGACTCATTATCCCTTGATAATGGAGAGAGCCAGACTAAATTT		
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          580      590      600      610      620      630
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gi|503  AAAGTTGGCCATGAAGACAGTGATTGGACAGCTTTCTCCTACTCAAATGCATCCTTCACA
          980      990      1000      1010      1020      1030

          640      650      660      670      680      690
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gi|503  CAATTGCTCAGTTTTGGAAAGGCCAAGAGTGGCTACTTTCTATCCATTTCTGATTCAAAA
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          700      710      720      730      740      750
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          940      950      960      970      980      990
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      1580      1590      1600      1610      1620      1630

      1240      1250      1260      1270      1280      1290
LEX41  ATCTATAGAATATCCGTAGTGATTGAGAACATCCTTCGTCACCCTGAGGTAAAAGTACAG
      .....
gi|503  ATCTATAGAATATCCGTAGTGATTGAGAACATCCTTCGTCACCCTGAGGTAAAAGTACAG
      1640      1650      1660      1670      1680      1690

      1300      1310      1320      1330      1340      1350
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gi|503  AGCAAGGTGGCAGAATGGCTCAATTCAACCTTCCAAAATTGGAACCTACACGGTTTATGTC
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LEX41  CCCAAAGGCTACTACTGGCCATCTATCCAACCTTCTGAATACGTTCTTCCTTGTCAGAC
gi|503  CCCAAAGGCTACTACTGGCCATCTATCCAACCTTCTGAATACGTTCTTCCTTGTCAGAC
      2000      2010      2020      2030      2040      2050

      1660      1670      1680      1690      1700      1710
LEX41  AAGCCTGGCTTTTCTGCTTCTCGGATATGTTTTTACAATGCTACCAACCCATTGGTAACC
gi|503  AAGCCTGGCTTTTCTGCTTCTCGGATATGTTTTTACAATGCTACCAACCCATTGGTAACC
      2060      2070      2080      2090      2100      2110

      1720      1730      1740      1750      1760      1770
LEX41  TACTGGGGACCTGTTGATATCTCCAACCTGTTTAAAAGAAGCAAATGAAGTTGCTAACCAG
gi|503  TACTGGGGACCTGTTGATATCTCCAACCTGTTTAAAAGAAGCAAATGAAGTTGCTAACCAG
      2120      2130      2140      2150      2160      2170

      1780      1790      1800      1810      1820      1830
LEX41  ATTTTAAATTTAACTGCTGATGGGCAGAACTTAACCTCAGCCAATATTACCAACATTGTG
gi|503  ATTTTAAATTTAACTGCTGATGGGCAGAACTTAACCTCAGCCAATATTACCAACATTGTG
      2180      2190      2200      2210      2220      2230

      1840      1850      1860      1870      1880      1890
LEX41  GAACAGGTCAAAGAATTGTGAATAAAGAAGAAAACATTGATATAACACTTGGCTCAACT
gi|503  GAACAGGTCAAAGAATTGTGAATAAAGAAGAAAACATTGATATAACACTTGGCTCAACT
      2240      2250      2260      2270      2280      2290

      1900      1910      1920      1930      1940      1950
LEX41  CTAATGAATATATTTTCTAATATCTTAAGCAGTTCAGACAGTGAAGTTGCTTGAGTCATCT
gi|503  CTAATGAATATATTTTCTAATATCTTAAGCAGTTCAGACAGTGAAGTTGCTTGAGTCATCT
      2300      2310      2320      2330      2340      2350

      1960      1970      1980      1990      2000      2010
LEX41  TCTGAAGCTTTAAAAACAATTGATGAATTGGCCTTCAAGATAGACCTAAATAGCACATCA
gi|503  TCTGAAGCTTTAAAAACAATTGATGAATTGGCCTTCAAGATAGACCTAAATAGCACATCA
      2360      2370      2380      2390      2400      2410

      2020      2030      2040      2050      2060      2070
LEX41  CATGTGAATATTACAACCTCGGAACCTGGCTCTCAGCGTATCATCCCTGTTACCAGGGACA
gi|503  CATGTGAATATTACAACCTCGGAACCTGGCTCTCAGCGTATCATCCCTGTTACCAGGGACA
      2420      2430      2440      2450      2460      2470

      2080      2090      2100      2110      2120      2130
LEX41  AATGCAATTTCAAATTTTAGCATTGGTCTTCCAAGCAATAATGAATCGTATTTCCAGATG
gi|503  AATGCAATTTCAAATTTTAGCATTGGTCTTCCAAGCAATAATGAATCGTATTTCCAGATG
      2480      2490      2500      2510      2520      2530

      2140      2150      2160      2170      2180      2190
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LEX41  GATTTTGAGAGTGGACAAGTGGATCCACTGGCATCTGTAATTTTGCCTCCAAACTTACTT
      .....
gi|503  GATTTTGAGAGTGGACAAGTGGATCCACTGGCATCTGTAATTTTGCCTCCAAACTTACTT
      2540      2550      2560      2570      2580      2590

      2200      2210      2220      2230      2240      2250
LEX41  GAGAATTTAAGTCCAGAAGATTCTGTATTAGTTAGAAGAGCACAGTTTACTTTCTTCAAC
      .....
gi|503  GAGAATTTAAGTCCAGAAGATTCTGTATTAGTTAGAAGAGCACAGTTTACTTTCTTCAAC
      2600      2610      2620      2630      2640      2650

      2260      2270      2280      2290      2300      2310
LEX41  AAAACTGGACTTTTCCAGGATGTAGGACCCCAAAGAAAAACTTTAGTGAGTTATGTGATG
      .....
gi|503  AAAACTGGACTTTTCCAGGATGTAGGACCCCAAAGAAAAACTTTAGTGAGTTATGTGATG
      2660      2670      2680      2690      2700      2710

      2320      2330      2340      2350      2360      2370
LEX41  GCGTGCAGTATTGGAAACATTACTATCCAGAATCTGAAGGATCCTGTTCAAATAAAAAATC
      .....
gi|503  GCGTGCAGTATTGGAAACATTACTATCCAGAATCTGAAGGATCCTGTTCAAATAAAAAATC
      2720      2730      2740      2750      2760      2770

      2380      2390      2400      2410      2420      2430
LEX41  AAACATACAAGAACTCAGGAAGTGCATCATCCCATCTGTGCCTTCTGGGATCTGAACAAA
      .....
gi|503  AAACATACAAGAACTCAGGAAGTGCATCATCCCATCTGTGCCTTCTGGGATCTGAACAAA
      2780      2790      2800      2810      2820      2830

      2440      2450      2460      2470      2480      2490
LEX41  AACAAAAGTTTTGGAGGATGGAACACGTCAGGATGTGTTGCACACAGAGATTCAGATGCA
      .....
gi|503  AACAAAAGTTTTGGAGGATGGAACACGTCAGGATGTGTTGCACACAGAGATTCAGATGCA
      2840      2850      2860      2870      2880      2890

      2500      2510      2520      2530      2540      2550
LEX41  AGTGAGACAGTCTGCCTGTGTAACCACTTCACACACTTTGGAGTTCTGATGGACCTTCCA
      .....
gi|503  AGTGAGACAGTCTGCCTGTGTAACCACTTCACACACTTTGGAGTTCTGATGGACCTTCCA
      2900      2910      2920      2930      2940      2950

      2560      2570      2580      2590      2600      2610
LEX41  AGAAGTGCCTCACAGTTAGATGCAAGAAACACTAAAGTCCTCACTTTCATCAGCTATATT
      .....
gi|503  AGAAGTGCCTCACAGTTAGATGCAAGAAACACTAAAGTCCTCACTTTCATCAGCTATATT
      2960      2970      2980      2990      3000      3010

      2620      2630      2640      2650      2660      2670
LEX41  GGGTGTGGAATATCTGCTATTTTTTTCAGCAGCAACTCTCCTGACATATGTTGCTTTTGAG
      .....
gi|503  GGGTGTGGAATATCTGCTATTTTTTTCAGCAGCAACTCTCCTGACATATGTTGCTTTTGAG
      3020      3030      3040      3050      3060      3070

      2680      2690      2700      2710      2720      2730
LEX41  AAATTGCGAAGGGATTATCCCTCCAAAATCTTGATGAACCTGAGCACAGCCCTGCTGTTC
      .....
gi|503  AAATTGCGAAGGGATTATCCCTCCAAAATCTTGATGAACCTGAGCACAGCCCTGCTGTTC
      3080      3090      3100      3110      3120      3130

      2740      2750      2760      2770      2780      2790
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LEX41 CTGAATCTCCTCTTCCTCCTAGATGGCTGGATCACCTCCTTCAATGTGGATGGACTTTGC
      .....
gi|503 CTGAATCTCCTCTTCCTCCTAGATGGCTGGATCACCTCCTTCAATGTGGATGGACTTTGC
      3140      3150      3160      3170      3180      3190

      2800      2810      2820      2830      2840      2850
LEX41 ATTGCTGTTGCAGTCCTGTTGCATTTCTTCCTTCTGGCAACCTTTACCTGGATGGGGCTA
      .....
gi|503 ATTGCTGTTGCAGTCCTGTTGCATTTCTTCCTTCTGGCAACCTTTACCTGGATGGGGCTA
      3200      3210      3220      3230      3240      3250

      2860      2870      2880      2890      2900      2910
LEX41 GAAGCAATTCACATGTACATTGCTCTAGTTAAAGTATTTAACACTTACATTCGCCGATAC
      .....
gi|503 GAAGCAATTCACATGTACATTGCTCTAGTTAAAGTATTTAACACTTACATTCGCCGATAC
      3260      3270      3280      3290      3300      3310

      2920      2930      2940      2950      2960      2970
LEX41 ATTCTAAAATTCTGCATCATTGGCTGGGGTTTGCCTGCCTTAGTGGTGTCAAGTTGTTCTA
      .....
gi|503 ATTCTAAAATTCTGCATCATTGGCTGGGGTTTGCCTGCCTTAGTGGTGTCAAGTTGTTCTA
      3320      3330      3340      3350      3360      3370

      2980      2990      3000      3010      3020      3030
LEX41 GCGAGCAGAAACAACAATGAAGTCTATGGAAAAGAAAGTTATGGGAAAGAAAAAGGTGAT
      .....
gi|503 GCGAGCAGAAACAACAATGAAGTCTATGGAAAAGAAAGTTATGGGAAAGAAAAAGGTGAT
      3380      3390      3400      3410      3420      3430

      3040      3050      3060      3070      3080      3090
LEX41 GAATTCTGTTGGATTCAAGATCCAGTCATATTTTATGTGACCTGTGCTGGGTATTTTGA
      .....
gi|503 GAATTCTGTTGGATTCAAGATCCAGTCATATTTTATGTGACCTGTGCTGGGTATTTTGA
      3440      3450      3460      3470      3480      3490

      3100      3110      3120      3130      3140      3150
LEX41 GTCATGTTTTTTTCTGAACATTGCCATGTTTCATTGTGGTAATGGTGCAGATCTGTGGGAGG
      .....
gi|503 GTCATGTTTTTTTCTGAACATTGCCATGTTTCATTGTGGTAATGGTGCAGATCTGTGGGAGG
      3500      3510      3520      3530      3540      3550

      3160      3170      3180      3190      3200      3210
LEX41 AATGGCAAGAGAAGCAACCGGACCCTGAGAGAAGAAGTGTTAAGGAACCTGCGCAGTGTG
      .....
gi|503 AATGGCAAGAGAAGCAACCGGACCCTGAGAGAAGAAGTGTTAAGGAACCTGCGCAGTGTG
      3560      3570      3580      3590      3600      3610

      3220      3230      3240      3250      3260      3270
LEX41 GTTAGCTTGACCTTTCTGTTGGGCATGACATGGGGTTTTGCATTCTTTGCCTGGGGACCC
      .....
gi|503 GTTAGCTTGACCTTTCTGTTGGGCATGACATGGGGTTTTGCATTCTTTGCCTGGGGACCC
      3620      3630      3640      3650      3660      3670

      3280      3290      3300      3310      3320      3330
LEX41 TTAAATATCCCCTTCATGTACCTCTTCTCCATCTTCAATTCATTACAAGGCTTATTTATA
      .....
gi|503 TTAAATATCCCCTTCATGTACCTCTTCTCCATCTTCAATTCATTACAAGGCTTATTTATA
      3680      3690      3700      3710      3720      3730

      3340      3350      3360      3370      3380      3390
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LEX41  TTCATCTTCCACTGTGCTATGAAGGAGAATGTTTCAGAAACAGTGGCGGCGGCATCTCTGC
      .....
gi|503  TTCATCTTCCACTGTGCTATGAAGGAGAATGTTTCAGAAACAGTGGCGGCGGCATCTCTGC
      3740      3750      3760      3770      3780      3790

      3400      3410      3420      3430      3440      3450
LEX41  TGTGGTAGATTTTCGGTTAGCAGATAACTCAGATTGGAGTAAGACAGCTACCAATATCATC
      .....
gi|503  TGTGGTAGATTTTCGGTTAGCAGATAACTCAGATTGGAGTAAGACAGCTACCAATATCATC
      3800      3810      3820      3830      3840      3850

      3460      3470      3480      3490      3500      3510
LEX41  AAGAAAAGTTCTGATAATCTAGGAAAATCTTTGTCTTCAAGCTCCATTGGTTCCAACTCA
      .....
gi|503  AAGAAAAGTTCTGATAATCTAGGAAAATCTTTGTCTTCAAGCTCCATTGGTTCCAACTCA
      3860      3870      3880      3890      3900      3910

      3520      3530      3540      3550      3560      3570
LEX41  ACCTATCTTACATCCAAATCTAAATCCAGCTCTACCACCTATTTCAAAAGGAATAGCCAC
      .....
gi|503  ACCTATCTTACATCCAAATCTAAATCCAGCTCTACCACCTATTTCAAAAGGAATAGCCAC
      3920      3930      3940      3950      3960      3970

      3580      3590      3600      3610      3620      3630
LEX41  ACAGACAGTGCTTCCATGGACAAGTCCTTGTCAAAACCTGGCCCCATGCTGATGGAGATCAA
      .....
gi|503  ACAGACAGTGCTTCCATGGACAAGTCCTTGTCAAAACCTGGCCCCATGCTGATGGAGATCAA
      3980      3990      4000      4010      4020      4030

      3640      3650      3660      3670      3680      3690
LEX41  ACATCAATCATCCCTGTCCATCAGGTCATTGATAAGGTCAAGGGTTATTGCAATGCTCAT
      .....
gi|503  ACATCAATCATCCCTGTCCATCAGGTCATTGATAAGGTCAAGGGTTATTGCAATGCTCAT
      4040      4050      4060      4070      4080      4090

      3700      3710      3720      3730      3740      3750
LEX41  TCAGACAACTTCTATAAAAAATATTATCATGTGCAGACACCTTCAGCCACAGCACAAAGTTT
      .....
gi|503  TCAGACAACTTCTATAAAAAATATTATCATGTGCAGACACCTTCAGCCACAGCACAAAGTTT
      4100      4110      4120      4130      4140      4150

LEX41  TAA
      ...
gi|503  TAATGTCTTTAAGAAAAAGAAATCAATCTGCAGAAATGTGAAGATTTGCAAGCAGTGTA
      4160      4170      4180      4190      4200      4210

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81.250% identity in 32 nt overlap (2878-2848:3249-3279)

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      2900      2890      2880      2870      2860
LEX41- ATCGGCGAATGTAAGTGTTAAATACTTTAACTAG-AGCAATGTACATGTGAATTGCTTCT
      .....
gi|503  TTCCTTCTGGCAACCTTTTACCTGGATGGGGCTAGAAGCAATTCACATGTACATTGC-TCT
      3220      3230      3240      3250      3260      3270

      2850      2840      2830      2820      2810      2800
LEX41- AGCCCCATCCAGGTAAAGGTTGCCAGAAGGAAGAAATGCAACAGGACTGCAACAGCAATG
      ..

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gi|503 AGTTAAAGTATTTAACACTTACATTGCGCGATACATTCTAAAATTCTGCATCATTGGCTG
      3280      3290      3300      3310      3320      3330
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3753 residues in 1 query sequences

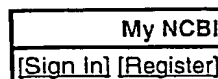
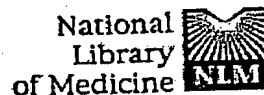
6902 residues in 1 library sequences

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Regulated expression of a vitellogenin fusion gene in transgenic nematodes.

Spieth J, MacMorris M, Broverman S, Greenspoon S, Blumenthal T.

Program in Molecular, Cellular, and Developmental Biology, Indiana University, Bloomington 47405.

In *Caenorhabditis elegans* the vitellogenin genes are expressed abundantly in the adult hermaphrodite intestine, but are otherwise silent. In order to begin to understand the mechanisms by which this developmental regulation occurs, we used the transformation procedure developed for *C. elegans* by A. Fire (EMBO. J., 1986, 5, 2673-2680) to obtain regulated expression of an introduced vitellogenin fusion gene. A plasmid with vit-2 upstream and coding sequences fused to coding and downstream sequences of vit-6 was injected into oocytes and stable transgenic strains were selected. We obtained seven independent strains, in which the plasmid DNA is integrated at a low copy number. All strains synthesize substantial amounts of a novel vitellogenin-like polypeptide of 155 kDa that accumulates in the intestine and pseudocoelom, but is not transported efficiently into oocytes. In two strains examined in detail the fusion gene is expressed with correct sex, tissue, and stage specificity. Thus we have demonstrated that the nematode transgenic system can give proper developmental expression of introduced genes and so can be used to identify DNA regulatory regions.

PMID: 3181632 [PubMed - indexed for MEDLINE]

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Feb 10 2005 12:03:04

Genetic transformation of mouse embryos by microinjection of purified DNA

(gene transfer/mice)

JON W. GORDON*, GEORGE A. SCANGOS†, DIANE J. PLOTKIN*, JAMES A. BARBOSA‡, AND FRANK H. RUDDLE**

*Department of Biology and †Department of Human Genetics, Yale University, New Haven, Connecticut 06511

Contributed by Frank H. Ruddle, September 23, 1980

ABSTRACT A recombinant plasmid composed of segments of herpes simplex virus and simian virus 40 viral DNA inserted into the bacterial plasmid pBR322 was microinjected into pronuclei of fertilized mouse oocytes. The embryos were implanted in the oviducts of pseudopregnant females and allowed to develop to term. DNA from newborn mice was evaluated by the Southern blotting technique for the presence of DNA homologous to the injected plasmid. Two of 78 mice in one series of injections showed clear homology, though the injected sequences had been rearranged. Band intensities from the two positive mice were consistent with the presence of donor DNA in most or all of the cells of the newborns. These results demonstrate that genes can be introduced into the mouse genome by direct insertion into the nuclei of early embryos. This technique affords the opportunity to study problems of gene regulation and cell differentiation in a mammalian system by application of recombinant DNA technology.

Introduction of specified gene sequences into mammalian embryos can be a powerful tool for the study of developmental genetic problems. The fate of such genes can be monitored throughout development by using sensitive probing techniques offered by recombinant DNA technology. In addition, the functioning of foreign genes in a normal host environment can be used to study the processes of gene regulation and to study the physiologic roles of products of such genes more precisely. Introduction of foreign DNA into all cells of an intact animal also provides an opportunity to pass sequences to offspring and to generate large numbers of transformed animals. In order to realize these benefits, it is necessary to transform embryos early in development and allow integration of foreign DNA into the cellular progenitors of the entire animal.

Such experiments with mammals are difficult. Zygotes must be maintained in culture conditions that at least grossly approximate the oviductal environment. Moreover, they can be maintained *in vitro* for only a few days, after which they must be returned to a female for implantation and further development. Insertion of material into early mammalian embryos is also difficult because of their small size.

Investigators have recently succeeded in constructing mosaic mice composed in part of descendants from cultured teratocarcinoma cells (1-3). This advance makes possible the introduction of genes into cultured cells, which might then be induced to cooperate in the formation of an intact adult mouse (4, 5). These cultured cells are often aneuploid, however, and some difficulty has been encountered in obtaining functional germ cells derived from them (6). Another problem with teratoma mosaics is that they are, indeed, mosaics. Thus, teratoma cells of XX chromosomal constitution cannot make sperm in

mice that develop as males; the possibility of germ-line transmission in this system is accordingly reduced. Jaenisch and Mintz (7) have provided evidence that whole DNA of simian virus 40 (SV40), when placed in cavities of mouse blastocysts, may be found in the resultant offspring. Ideally, however, one would like to introduce a small amount of well-defined genetic material directly into normal embryos and allow this material to integrate and function within the host genome.

We have approached this problem by injecting DNA directly into the pronuclei of fertilized mouse oocytes. The one-cell stage was chosen in order to limit as much as possible the development of mosaicism during cleavage. To avoid the hazards of culture, injected embryos were immediately implanted into the oviducts of pseudopregnant recipients. The DNA chosen for injection was the bacterial plasmid pBR322 into which had been inserted fragments of herpes simplex and SV40 viral DNA. This plasmid was constructed because the SV40 fragment is known to contain an origin of DNA replication, whereas the herpes fragment codes for a gene product, thymidine kinase (TK), distinguishable from the endogenous mouse enzyme. DNA was extracted from newborn mice and screened by the Southern blotting technique for the presence of sequences homologous to the injected plasmid. Two of 78 mice evaluated in one experimental series were found to contain such sequences. In both instances the injected DNA had been modified, but it could be demonstrated to be derived from donor material. The intensity of the positive bands indicated that an amount of DNA roughly equivalent to one copy in every cell of the newborns was retained. We thus provide evidence that mice can be genetically transformed by direct insertion of DNA into early embryos.

MATERIALS AND METHODS

Mice. CD-1 mice were obtained from the Charles River Breeding Laboratories. B6D2F₁ mice were obtained from the Jackson Laboratory. All mice were maintained on a 14:10 light-dark schedule (lights off at 10 p.m., on at 8 a.m.). Six-week-old females were induced to superovulate with 5 international units of pregnant mares' serum (Gestyl, Organon) at 4 p.m. followed 48 hr later by 2.5 international units of human chorionic gonadotropin (Pregnyl, Organon) and placed immediately with males for mating. B6D2F₁ females were mated with CD-1 males; CD-1 females were mated with B6D2F₁ males. On the same evening other mature CD-1 females were placed with vasectomized CD-1 males. On the morning after mating (day 0) all female mice were examined for vaginal plugs. Six-week-old females were killed at 2 p.m. on day 0 and

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Abbreviations: SV40, simian virus 40; TK, thymidine kinase; kb, kilobase(s).

† Present address: Department of Biology, The Johns Hopkins University, Baltimore, MD 21218.

their oviducts were removed into Krebs-Ringer bicarbonate-buffered medium supplemented with bovine serum albumin (8) and hyaluronidase at 1 mg/ml. Oviducts were opened with forceps and the fertilized eggs with remaining follicle cells were expressed into the dish. After 1–2 min, eggs were removed and washed three times in 2 ml of culture medium equilibrated with 5% CO₂ in air at 37°C. Eggs containing pronuclei were identified under the dissecting microscope and placed in lots of 20 in a microdrop of equilibrated medium, which was placed in a 100-mm tissue culture dish and covered with mineral oil (Mallinckrodt 6358). Eggs were stored in this manner in the incubator until microinjected.

Microinjection. Microneedles were pulled from thin-walled no. 1211L Omega Dot tubing (Glass Co. of America) on a DK1 model 700C pipette puller. Holding pipettes were pulled by hand on a microburner from G-12 capillary tubing (Thomas), and fire polished on a Sensar microforge. The tips of the microneedles were allowed to fill with plasmid suspension by capillary action and the barrels were then filled with Fluorinert (3M FC77). They were then secured in PE-190 intramedic tubing on a Leitz micromanipulator. Holding pipettes were also filled with Fluorinert and similarly secured in PE-90 tubing. The tubing was likewise filled with Fluorinert and attached to 1-cm³ Hamilton syringes. All manipulations were carried out on a Leitz microscope.

Tissue culture dishes containing the fertilized eggs were placed on the microscope and eggs were positioned by holding the pipette such that a pronucleus near the plasma membrane was close to the microneedle. The microneedle was inserted into the pronucleus and enough plasmid suspension was injected to cause an approximate doubling of the pronuclear volume (approximately 1 pl). Eggs that survived microinjection were removed and stored in a 30-mm tissue culture dish containing 2 ml of equilibrated medium until all microinjections were completed. Injection of 40–60 embryos required 1–2 hr.

Implantation. Plugged pseudopregnant CD-1 females were anesthetized with Nembutal at 6 mg/100 g of body weight. Ovaries were located through a dorsal incision. The ovarian bursa was torn away with no. 5 Dumont watchmaker's forceps, taking care not to rupture large blood vessels. The ostium of the oviduct was visualized under the dissecting microscope and a pipette containing 10–20 microinjected embryos was inserted into it. The eggs were expelled into the oviduct and the wound was closed with wound clips. Mice were examined on days 18–21 for the delivery of live offspring. Newborn mice were stored at –80°C for later analysis. Sixty percent of the embryos survived microinjection; 30–50% of the survivors developed into live young. All newborns were normal in appearance. All microinjection work was carried out under P1 containment in accordance with National Institutes of Health guidelines.

DNA Isolation. DNA was isolated from whole newborn mice by the method of Blin and Stafford (9) with the following modifications. Powdered tissue was incubated for 4 hr at 50°C in 22 ml of 0.28 M EDTA/0.5% Sarkosyl, pH 7.0. The homogenate was subsequently extracted twice in phenol/chloroform/isoamyl alcohol (15 ml:5 ml:0.2 ml), and once in chloroform/isoamyl alcohol (15 ml:0.6 ml). The extract was dialyzed for 24 hr against 10 mM Tris-HCl, pH 8.0/10 mM NaCl/1 mM EDTA and precipitated with a 2-vol excess of 100% ethyl alcohol. Precipitated DNA was stored at –20°C until use.

Filter Hybridization. DNA was redissolved in 1× TEN (10 mM Tris-HCl, pH 7.75/10 mM NaCl/0.1 mM EDTA) to yield a final concentration of approximately 1 mg/ml. Twenty micrograms of DNA was digested at a 10- to 20-fold excess with appropriate restriction enzymes (Bethesda Research Laboratories, Rockville, MD). After overnight digestion at 37°C,

samples were electrophoresed in 1% agarose in 160 mM Tris-HCl/80 mM NaOAc/80 mM NaCl/5 mM EDTA, pH 8, at 350 A for 22 hr. Samples were then blotted onto nitrocellulose filters according to the method of Southern (10).

Nick translations were performed by using the New England Nuclear nick translation kit with ³²P-labeled dCTP obtained from New England Nuclear. Filter hybridizations were performed as described by Wahl *et al.* (11). Filters were then used to expose Kodak X-Omat x-ray film, using intensifying screens, until band intensities were appropriate for analysis.

Construction of the Plasmid. The recombinant plasmids, called pST6, pST9 and pST12, carrying the SV40 origin of replication and promoters, and the herpes simplex virus TK gene were constructed by inserting the SV40 *Hind*III-C fragment (12, 13) into the available *Hind*III site in the plasmid pTKX-1 (14). DNA from the SV40 mutant 1265, kindly provided by C. Cole of Yale University, was digested to completion with restriction enzymes *Hind*III and *Hinf*I (New England BioLabs) simultaneously. The double digestion generated two fragments larger than 550 base pairs; the *Hind*III-C fragment (1099 base pairs; map position 0.649–0.859) and the *Hinf*I-B fragment (1085 base pairs; 0.992–0.199), which comigrated on a 1% Seaplaque agarose gel. The 1.1-kilobase (kb) doublet band was extracted from the gel and ligated with pTKX-1 that had been digested with *Hind*III and alkaline phosphatase [as described by Ullrich *et al.* (15) except that bovine alkaline phosphatase (Sigma) was used]. The molar ratio of the vector to target in the ligation mixture was 3:1. The ligation mixture was incubated at 4°C for 17 hr with one addition of phage T4 ligase at 11 hr. The mixture was used to transform *Escherichia coli* strain HB 101, and ampicillin-resistant colonies were selected. Colonies carrying the putative pST plasmids were identified by colony hybridization (16), using SV40 DNA as the probe. Approximately 20% of the ampicillin-resistant colonies contained SV40 sequences. Confirmation of the *Hind*III-C fragment insertion and determination of its orientation in the plasmid was done by restriction analysis of mini-DNA isolations (17). A restriction endonuclease map of the plasmid pST6 is shown in Fig. 1. This work was carried out under P2 containment in accordance with National Institutes of Health guidelines.

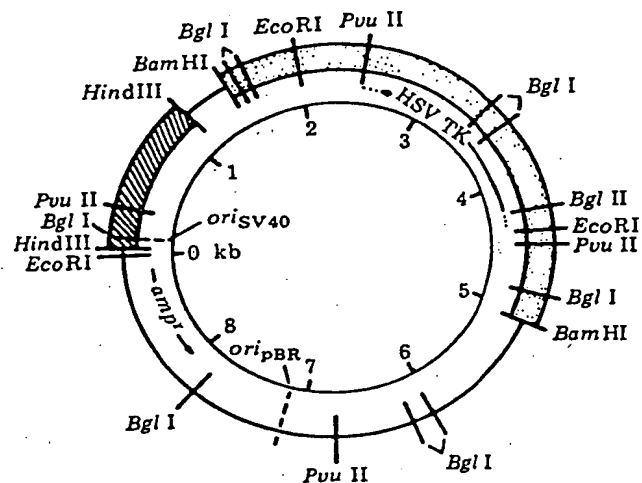


FIG. 1. The circular plasmid pST6, a derivative of pBR322. Hatched area shows the SV40 insert; stippled area denotes the herpes simplex virus TK insert. *amp*^r, ampicillin resistance gene; *ori*, origin of DNA replication.

Table 1. Summary of microinjection data

Exp.	Plasmid	Copies injected per cell	Offspring	Plasmid DNA positives
1	pST6	1,000	78	2
2	pST6	12,000	10	0
3	pST6 (linearized)	1,000	40	0
4	pST9	1,000	16	0
5	pRH 1.3Mm 1	1,000	27	0
6	pST12	500	2	0
7	Uninjected control	—	54	0

The pRH 1.3Mm 1 plasmid consists of a cloned fragment of a member of the highly repeated and interspersed *EcoRI*-*Bgl* II sequence family cloned in pBR322, provided by N. Arnheim (18). pST9 is identical to pST6, except that the orientation of the SV40 fragment is reversed. pST12 is a dimer of pST6. pST was linearized by *Sac* I digestion. A total of 187 mice were born from microinjected embryos.

RESULTS

Results of the plasmid microinjections are summarized in Table 1. In the first experimental series, injection of several hundred embryos yielded 78 live young. DNA was extracted from whole newborn mice for rapid and efficient determination of transformation frequency. The screening method gives a low estimate of the number of transformants; embryos with transforming DNA in a small percentage of their cells could have escaped detection. DNA from 2 of these 78 newborn mice contained sequences that hybridized strongly with the probe, pST6. The restriction endonuclease patterns of the incorporated sequences were significantly different between the two offspring, and are described below.

DNA from the first positive animal, no. 48, gave two intense bands with estimated sizes of 12.9 kb and 9.8 kb and a third band of very large size (>24 kb) when digested with *Bam*HI (Fig. 2). The positions of the two smaller bands were unaffected by digestion with *Hind*III, *Eco*RI, *Bam*HI, or *Xba*I (Fig. 2). This result suggested that the TK sequences, which had been inserted into the *Bam*HI sites, and the SV40 sequences inserted into the *Hind*III sites were not present in their native state in the incorporated material. The *Hind*III digestion, however, was incomplete as judged from the control track. We therefore probed with SV40 DNA alone. No sequences homologous to this

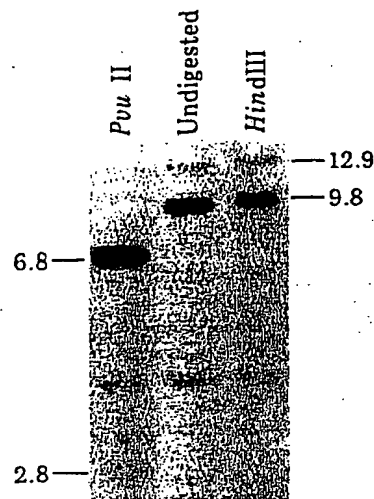


FIG. 3. DNA from mouse no. 48 digested with *Hind*III or *Pou* II, or undigested; probed with pST6. Fragment sizes are indicated in kb.

probe were detected. The 12.9- and 9.8-kb fragments appeared in the undigested sample, consistent with their presence as free molecules. Digestion of the DNA with *Pou* II generated two bands of altered mobility, 2.8 kb and 6.8 kb in size (Fig. 3). This result indicated that the sequences represented by the 12.9- and 9.8-kb bands contained at least one *Pou* II site. We believe these results, taken together, are consistent with the existence of free circular molecules in the DNA of mouse no. 48.

The second positive, no. 73, showed a markedly different blotting pattern. In the undigested DNA, hybridizable material was not separable from the high molecular weight mouse DNA. Moreover, digestion with *Xba*I, which does not cut pST6, gave a single band of greater size than the highest molecular weight standard of 23.7 kb. Finally, several bands showed homology with probes synthesized from either purified SV40 DNA or TK fragment (Fig. 4). Thus, this animal had retained all or part of these portions of the plasmid.

Digestion with *Bam*HI yielded three major bands, 7.8 kb, 3.9 kb, and 3.4 kb. The largest band showed homology with

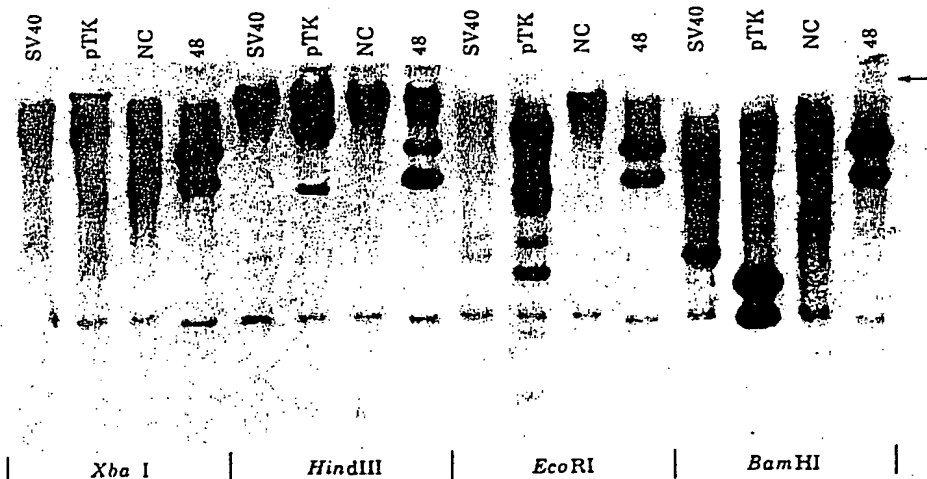


FIG. 2. DNA from mouse no. 48 digested with *Bam*HI, *Eco*RI, *Hind*III, and *Xba*I. The labeled probe was pST6 DNA. NC indicates the negative control (DNA isolated from uninjected mice). Positive controls include (i) NC DNA with SV40 DNA added (SV40) and (ii) NC DNA with the plasmid pTTX-1 added (pTK). Arrow indicates the high molecular weight band that appears reproducibly in *Bam*HI digests.

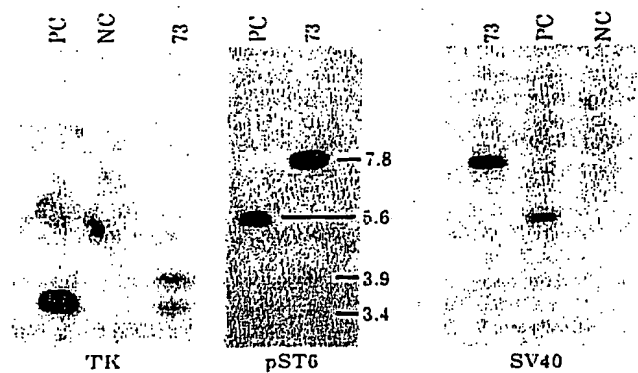


FIG. 4. DNA from mouse no. 73 digested with *Bam*HI and probed with pST6 (Center), SV40 DNA (Right), or TK fragment (Left). Positive control (PC) consists of pST6 added to mouse DNA to a concentration of 10^{-8} by weight. NC denotes the negative control (DNA isolated from uninjected mice).

SV40, SV40 + pBR322, and with the whole plasmid, pST6 (Fig. 4). The two smaller bands showed homology with TK fragment, but not with SV40 or pBR322 (Fig. 4). The probeable portions of these smaller pieces were thus composed entirely of TK-derived material. The smallest band, 3.4 kb, closely approximates the size of the TK fragment that was inserted into the *Bam*HI sites, suggesting that the entire TK gene had been retained in no. 73. Digestion with *Bgl* I, however, proved this supposition incorrect. An internal fragment of TK approximately 1.8 kb in size, defined by *Bgl* I sites, did not appear in the DNA (data not shown). This showed that the 3.4- and 3.9-kb *Bam*HI fragments were composed of portions of the TK fragment that had either been concatamerized or complexed with mouse DNA to yield molecular weights equal to or greater than the molecular weight of the original TK insert.

Digests with *Pvu* II and *Hind*III provided strong evidence that the entire SV40 sequence was retained. Digestion with *Hind*III produced a fragment very close in size to the SV40 insert of pST6. In addition, digestion with *Pvu* II gave two fragments that migrated indistinguishably from the *Pvu* II-defined SV40 fragments of pST6. Thus, two independent experiments support the contention that the entire SV40 fragment was present.

DISCUSSION

These data demonstrate that it is possible to use a recombinant plasmid as a vector for transfer of foreign genes directly into mouse embryos, and that these embryos can maintain the foreign genes throughout development. Moreover, the intensity of the bands on Southern blot analysis suggests that most or all of the cells of the newborns contained derivatives of the injected plasmid. Blotting experiments with hybrid cell populations have shown that sequences cannot be detected if present in fewer than 10% of the cells (19). We are thus confident that the two transformed mice contained enough plasmid DNA for distribution of one copy to at least this percentage of their cells. Our positive controls were adjusted to correspond to one copy of pST6 per diploid genome. The band intensities of no. 48 and no. 73 are comparable to the control. Thus, the transforming sequences are probably present in far greater amounts than the 10% threshold of detectability; the band intensities are more consistent with the presence of the plasmid derivative in most or all of the cells of the newborns. Our method of analysis cannot rule out the possibility that only a few of the cells contained all of the sequences while most of the cells were negative, but we consider unlikely the chances that cells carrying a large

amount of additional genetic material would survive and compete successfully through development. If the transforming sequences were in fact distributed throughout the tissues of the mice, then integration must have occurred at an early stage, shortly after determination of the inner cell mass. Injection of one-celled embryos may be important for obtaining early integration. In addition, the high mortality caused by microinjection suggests that injection of only a fraction of the cells of a later cleavage stage might result in preferential survival of uninjected blastomeres and consequently give a lower rate of success.

The transformation rate reported here compares very favorably with other gene transfer systems involving mammalian cells. Calcium phosphate-mediated gene transfer into cultured cells results in transformation rates of 10^{-8} to 10^{-5} (20, 21), while microinjection of cultured cells gives approximately 5% success (22). Our transformation rate agrees well with these latter results. The reasons for higher rates in microinjection experiments are unknown but may include the facts that DNA is inserted directly into the nucleus and that gene expression is not required in the mouse system.

Significant differences were found between the two transformed mice. In mouse no. 48, SV40 and herpes viral TK DNA could not be detected. The remaining sequences, derived from pBR322, were complexed into three bands, all of higher molecular weight than the entire pBR322 plasmid. In addition, two of these bands represented DNA that probably existed free of the host genome. The presence of unintegrated sequences in no. 48 is intriguing. Two plausible models can be invoked to explain this observation: (i) these sequences may have replicated autonomously and persisted as plasmid-like units; (ii) alternatively, they may have been generated from an integrated segment. The former model requires that the free sequences have the capacity to replicate. The plasmid from which they descended did contain the pBR322 and SV40 origins. But, interestingly, SV40 DNA is undetectable in the retained material. It is also possible that a mouse origin was acquired as a result of interaction with the host genome.

It is more likely that the free sequences were generated from integrated material. Generation of free circular DNA from transformed cultured cells has been observed previously (23). Cells infected with viruses can also generate free DNA from the integrated viral genome (24). In addition, cells transformed in calcium phosphate-mediated gene transfer experiments can pass through an unstable phase during which the donated material is maintained independent of the host genome as high molecular weight "transgenomes" (25). An important characteristic of these independent transgenomes is their rapid loss from recipient cells; as many as 10% of the cells may lose the transforming sequences per day (25). The rearrangement of the donor material in no. 48 appears analogous to transgenome formation in cultured cells. If the unintegrated sequences were similar to independent transgenomes, we would expect them to be rapidly lost from the mouse cells during development and not detectable in the newborn. The marked intensity of the two bands in no. 48 rather suggests that they were continuously being produced from an integrated sequence. The presence of a high molecular weight band after digestion with *Bam*HI is also consistent with the integration model. This band may represent material from which the two smaller bands were generated.

In mouse no. 73, no free sequences were present. Both the undigested and *Xba* I-digested samples gave single bands of greater size than the highest molecular weight standard. Moreover, SV40 and TK sequences were retained in this animal. The patterns of bands present in mouse no. 73 is explained best

by plasmid integration into the mouse genome at a site within the TK region. In this model, digestion of the mouse DNA with *Bam*HI would generate three plasmid-derived fragments, two of which would consist of the TK fragment (now at both ends of the integrated molecule) linked to mouse DNA. The third fragment would be cleaved from within the integrated plasmid and would contain the SV40 and pBR322 moieties. The predicted size of this internal fragment is 5.5 kb. This model also predicts that the TK fragment would be disrupted and that the SV40 and pBR322 sequences would be intact. The DNA of mouse no. 73 contained two bands of 3.4 and 3.9 kb that hybridized only with the purified TK fragment and contained no sequences homologous to SV40 or pBR322, and a band of 7.8 kb that hybridized to SV40 and not to TK. The large size of this fragment relative to the expected 5.5-kb fragment might be due to partial internal duplication, which is consistent with independent observations of SV40 integration (26, 27). Digestion of the DNA of mouse no. 73 with *Bgl*I or with *Pvu*II failed to generate expected fragments from within the TK insert but indicated that most or all of pBR322 and SV40 were present. Additionally, *Hind*III digestion generated a band of the expected size of the SV40 insert, indicating that all of the SV40 sequences present on pST6 were also present in the DNA of mouse no. 73 (data not shown). Thus, our observations are consistent with a single integration event.

An important similarity between the two positive mice was the extensive rearrangement of the sequences. In the first instance, SV40 and herpes virus TK sequences were largely if not entirely removed from the injected DNA. In the second case, SV40 sequences and herpes virus TK sequences were demonstrable, but the TK gene was significantly rearranged. These observations raise the possibility that selection occurred against embryos that retained the TK gene intact and in an active state. The possibility that herpes virus TK is teratogenic to mouse embryos is consistent with our data. We consider this notion unlikely, however, because cells transformed in culture and under selection for TK demonstrate similar patterns of rearrangement (25, 28).

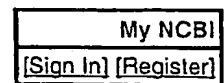
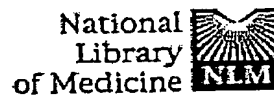
These initial results show that genetic transformation can be extended to whole mammalian organisms at a very early stage in their development. Further refinement of these techniques should lead to a reliable system of embryo transformation with its attendant applications for investigation of problems in development and cell differentiation.

Note Added in Proof. We have produced a third transformant by injection of 30,000 copies per cell of the plasmid pST9. Restriction analysis indicates that, as in mouse no. 73, the transforming sequences are integrated. Initial studies also indicate that at least one complete copy each of both the herpes virus TK and SV40 regions has been retained in this animal.

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1. Brinster, R. L. (1974) *J. Exp. Med.* 140, 1049-1056.
2. Mintz, B. & Illmensee, K. (1975) *Proc. Natl. Acad. Sci. USA* 72, 3585-3589.
3. Papaioannou, V. E., McBurney, M. W., Gardner, R. L. & Evans, M. S. (1975) *Nature (London)* 258, 69-73.
4. Illmensee, K., Hoppe, P. C. & Croce, C. M. (1978) *Proc. Natl. Acad. Sci. USA* 75, 1914-1918.
5. Pellicer, A., Wagner, E. F., Karem, A. E., Dewey, M. J., Reuser, A. J., Silverstein, S., Axel, R. & Mintz, B. (1980) *Proc. Natl. Acad. Sci. USA* 77, 2098-2102.
6. Illmensee, K. (1978) in *Genetic Mosaics and Chimeras in Mammals*, ed., Russell, L. B. (Plenum, New York), pp. 3-25.
7. Jaenisch, R. & Mintz, B. (1974) *Proc. Natl. Acad. Sci. USA* 71, 1250-1254.
8. Mullen, R. J. & Whitten, W. K. (1971) *J. Exp. Zool.* 178, 165-176.
9. Blin, N. & Stafford, D. W. (1976) *Nucleic Acids Res.* 3, 2303-2308.
10. Southern, E. M. (1975) *J. Mol. Biol.* 98, 503-517.
11. Wahl, G. M., Stern, M. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* 76, 3683-3687.
12. Reddy, V. B., Thimmappaya, B., Dahr, R., Subramanian, K. N., Zain, B. S., Pan, J., Ghosh, P. K., Celma, B. L. & Weissmann, S. W. (1978) *Science* 200, 494-502.
13. Fiers, W., Contreras, R., Haegeman, G., Rogiers, R., Van de Voorde, A., Van Heuverswyn, A., Van Herreweghe, J., Volckaert, G. & Ysebaert, M. (1978) *Nature (London)* 273, 113-120.
14. Enquist, L. W., Van de Woude, G. F., Wagner, M., Smiley, J. R. & Summers, W. C. (1979) *Gene* 7, 335-342.
15. Ullrich, A., Shine, J., Chirgwin, J., Pictet, R., Tischer, E., Rutter, W. J. & Goodman, H. M. (1977) *Science* 196, 1313-1319.
16. Gergen, J. P., Stern, R. H. & Wensink, P. C. (1979) *Nucleic Acids Res.* 7, 2115-2136.
17. Birnboim, H. C. & Doty, J. (1979) *Nucleic Acids Res.* 7, 1513-1523.
18. Heller, R. & Arnheim, N. (1980) *Nucleic Acids Res.*, in press.
19. D'Eustachio, P., Pravtcheva, D., Marcu, K. & Ruddle, F. H. (1980) *J. Exp. Med.* 151, 1545-1550.
20. Wigler, M., Pellicer, A., Silverstein, S. & Axel, R. (1978) *Cell* 14, 725-731.
21. Lester, S. C., LeVan, S. K., Steglich, C. & DeMars, R. (1980) *Somatic Cell Genet.* 6, 241-259.
22. Anderson, W. F., Killos, L., Sanders-Haigh, L., Kretschmer, P. J. & Diacumakos, E. G. (1980) *Proc. Natl. Acad. Sci. USA* 77, 5399-5403.
23. Schegget, J. T., Voves, J., Strien, A. V. & Van der Noordaa, J. (1980) *J. Virol.* 35, 331-339.
24. Hanahan, D., Lane, D., Lipsich, L., Wigler, M. & Botchan, M. (1980) *Cell* 21, 127-140.
25. Scangos, G. A., Huttner, K. M., Juricek, D. K. & Ruddle, F. H., *Mol. Cell Biol.*, in press.
26. Kelly, T. J., Jr., Lewis, A. M., Jr., Levine, A. S. & Siegel, S. (1974) *J. Mol. Biol.* 89, 113-126.
27. Botchan, M., Topp, W. & Sambrook, J. (1979) *Cold Spring Harbor Symp. Quant. Biol.* 43, 709-719.
28. Huttner, K. M., Scangos, G. A. & Ruddle, F. H. (1979) *Proc. Natl. Acad. Sci. USA* 76, 5820-5824.



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1: Nature. 1990 Apr 5;344(6266):541-4.

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Fulminant hypertension in transgenic rats harbouring the mouse Ren-2 gene.

Mullins JJ, Peters J, Ganten D.

German Institute for High Blood Pressure Research, University of Heidelberg.

PRIMARY hypertension is a polygenic condition in which blood pressure is enigmatically elevated; it remains a leading cause of cardiovascular disease and death due to cerebral haemorrhage, cardiac failure and kidney disease. The genes for several of the proteins involved in blood pressure homeostasis have been cloned and characterized, including those of the renin-angiotensin system, which plays a central part in blood pressure control. Here we describe the introduction of the mouse Ren-2 renin gene into the genome of the rat and demonstrate that expression of this gene causes severe hypertension. These transgenic animals represent a model for hypertension in which the genetic basis for the disease is known. Further, as the transgenic animals do not overexpress active renin in the kidney and have low levels of active renin in their plasma, they also provide a new model for low-renin hypertension.

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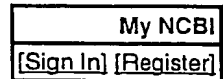
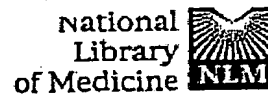
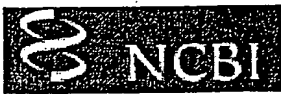
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Production of transgenic rabbits, sheep and pigs by microinjection.

Hammer RE, Pursel VG, Rexroad CE Jr, Wall RJ, Bolt DJ, Ebert KM, Palmiter RD, Brinster RL.

Direct microinjection has been used to introduce foreign DNA into a number of terminally differentiated cell types as well as embryos of several species including sea urchin, *Candida elegans*, *Xenopus*, *Drosophila* and mice. Various genes have been successfully introduced into mice including constructs consisting of the mouse metallothionein-I (MT) promoter/regulator region fused to either the rat or human growth hormone (hGH) structural genes. Transgenic mice harbouring such genes commonly exhibit high, metal-inducible levels of the fusion messenger RNA in several organs, substantial quantities of the foreign growth hormone in serum and enhanced growth. In addition, the gene is stably incorporated into the germ line, making the phenotype heritable. Because of the scientific importance and potential economic value of transgenic livestock containing foreign genes, we initiated studies on large animals by microinjecting the fusion gene, MT-hGH, into the pronuclei or nuclei of eggs from superovulated rabbits, sheep and pigs. We report here integration of the gene in all three species and expression of the gene in transgenic rabbits and pigs.

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RESEARCH PAPERS

Bio/Technology 8, 140 - 143 (1990)
doi:10.1038/nbt0290-140**Rabbit β -Casein Promoter Directs Secretion of Human Interleukin-2 into the Milk of Transgenic Rabbits**Th. A. Bühler¹, Th. Bruyère², D. F. Went¹, G. Stranzinger¹ & K. Bürki²¹Swiss Federal Institute of Technology Zürich, Institute for Animal Science, CH-8092 Zürich.²Preclinical Research, Sandoz Ltd., CH-4002 Basel.

To test the potential usefulness of transgenic rabbits as production systems for human proteins of pharmaceutical value, we cloned the rabbit β -casein promoter and fused it to the genomic sequence of the human interleukin-2 (hIL2) gene. Four transgenic female rabbits were tested for expression and biological activity of the foreign protein in their milk. The milk of all four females proved to contain biologically active hIL2. The results show that transgenic rabbits may represent a convenient and economic system for the rapid production of biologically active protein in milk.

REFERENCES

- Gordon, K., Lee, E., Vitale, J.A., Smith, A.E., Westphal, H. and Hennighausen, L. 1987. Production of human tissue plasminogen activator in transgenic mouse milk. *Bio/Technology* 5: 1183-1187. | [ISI](#) | [ChemPort](#) |
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3. Clark, A.J., Bessos, H., Bishop, J.O., Brown, P., Harris, S., Lathe, R., McCenaghan, M., Prowse, C., Simons, J.P., Whitelaw, C.B.A. and Wilmut, I. 1989. Expression of human anti-hemophilic factor IX in the milk of transgenic sheep. *Bio/Technology* **7**: 487–492. | [ISI](#) | [ChemPort](#) |
 4. Simons, J.P., Wilmut, L., Clark, A.J., Archibald, A.L., Bishop, J.O. and Lathe, R. 1988. Gene transfer into sheep. *Bio/Technology* **6**: 179–183. | [ISI](#) | [ChemPort](#) |
 5. Van Brunt, J. 1988. Molecular farming: Transgenic animals as bio-reactors. *Bio/Technology* **6**: 1149–1154.
 6. Vakulenko, I.S. 1984. Milk yield of rabbits and the growth of their young. *Krolikovodstvo i Zverovodstvo* **5**: 11–13.
 7. Cowie, A.T. 1969. Variations in the yield and composition of the milk during lactation in the rabbit and the galactopoietic effect of prolactin. *J. Endocr.* **44**: 437–450. | [PubMed](#) | [ISI](#) | [ChemPort](#) |
 8. Jeness, R. and Sloan, R.E. 1970. The composition of milks of various species—a review. *Dairy Sci. Abstr.* **32**: 599–612.
 9. Dayal, R., Hurlimann, J., Suard, M.L. and Kraehenbuhl, J-P. 1982. Chemical and immunochemical characterization of caseins and the major whey proteins of rabbit milk. *Biochem. J.* **201**: 71–79. | [PubMed](#) | [ISI](#) | [ChemPort](#) |
 10. Bühler, Th.A., Bruyère, Th. and Bürki, K. 1989. Nucleotide sequence of the rabbit β -casein 5' flanking region. EMBL database No. X15735.
 11. Degraeve, W., Tavernier, J., Duerink, F., Plaetinck, G., Devos, R. and Fiers, W. 1983. Cloning and structure of the human interleukin-2 chromosomal gene. *EMBO J.* **2**: 2349–2353. | [PubMed](#) | [ISI](#) | [ChemPort](#) |
 12. Smith, K.A., 1988. Interleukin-2: Inception, impact and implications. *Science* **240**: 1169–1176. | [PubMed](#) | [ISI](#) | [ChemPort](#) |
 13. Sanger, F., Nicklen, S. and Coulson, A.R. 1977. DNA sequencing with chain termination inhibitors. *Proc. Natl. Acad. Sci. USA* **74**: 5463–5467. | [PubMed](#) | [ChemPort](#) |
 14. Jones, W.K., Yu-Lee, L., Clift, S.M., Brown, T.L. and Rosen, J.M. 1985. The rat casein multigene family. *J. Biol. Chem.* **260**: 7042–7050. | [PubMed](#) | [ISI](#) | [ChemPort](#) |
 15. Gorodetsky, S.I., Tkach, T.M. and Kapelinskaya, T.V. 1988. Isolation and characterization of the *Bos taurus* β -casein gene. *Gene* **66**: 87–96. | [Article](#) | [PubMed](#) | [ISI](#) | [ChemPort](#) |
 16. Yu-Lee, L., Richter-Mann, L., Couch, C.H., Stewart, A.F., Mackinlay, A.G. and Rosen, J.M. 1986. Evolution of the casein multigene family: conserved sequences in the 5' flanking and exon regions. *Nucl. Acids Res.* **14**: 1883–1902. | [PubMed](#) | [ChemPort](#) |
 17. Scheidereit, C., Westphal, H., Carlson, C., Bosshard, H. and Beato, M. 1986. Molecular model of the interaction between the glucocorticoid receptor and the regulatory elements of

- inducible genes. *DNA* **5**: 383-391. | [PubMed](#)
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18. Brinster, R.L., Chen, H.Y., Trumbauer, M.E., Yagle, M.K. and Palmiter, R.D. 1985. Factors affecting the efficiency of introducing foreign DNA into mice by microinjecting eggs. *Proc. Natl. Acad. Sci. U.S.A.* **82**: 4438-4442. | [PubMed](#)
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 19. Hammer, R.E., Pursel, V.G., Rexroad, C.E., Wall, R.J., Bolt, D.J., Ebert, K.M., Palmiter, R.D. and Brinster, R.L. 1985. Production of transgenic rabbits, sheep and pigs by microinjection. *Nature* **315**: 680-683. | [PubMed](#)
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 20. Robb, R.J., 1982. Human T-cell growth factor: Purification, biochemical characterisation, and interaction with a cellular receptor. *Immunobiol.* **161**: 21-50. | [ISI](#) | [ChemPort](#) |
 21. Lebas, F. 1970. Description d'une machine à traire les lapins. *Ann. Zootech.* **19**: 223-228. | [ISI](#) |
 22. Lee, K.F., Atiee, S.H. and Rosen, J.M. 1989. Differential regulation of rat β -casein-chloramphenicol acetyltransferase fusion gene expression in transgenic mice. *Mol. Cell. Biol.* **9**: 560-565. | [PubMed](#) | [ISI](#) | [ChemPort](#) |
 23. Bornstein, P., McKay, J., Liska, D.J., Apone, S. and Devarayalu, S. 1988. Interactions between the promoter and the first intron are involved in transcriptional control of $\alpha 1(I)$ collagen gene expression. *Mol. Cell. Biol.* **8**: 4851-4857. | [PubMed](#) | [ISI](#) | [ChemPort](#) |
 24. Lee, K.F., DeMayo, F.J., Atiee, S.H. and Rosen, J.M. 1988. Tissue specific expression of the rat β -casein gene in transgenic mice. *Nucl. Acids Res.* **16**: 1027-1041. | [PubMed](#)
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 25. Grosveld, F., van Assendelft, G.B., Greaver, D.R. and Kollias, G. 1987. Position-independent, high-level expression of the human β -globin gene in transgenic mice. *Cell* **51**: 975-985. | [Article](#) | [PubMed](#) | [ISI](#) | [ChemPort](#) |
 26. Talbot, D., Collis, P., Antoniou, M., Vidal, M., Grosveld, F. and Greaves, D.R. 1989. A dominant control region from the human β -globin locus conferring integration site-independent gene expression. *Nature* **338**: 352-355. | [Article](#) | [PubMed](#) | [ISI](#) | [ChemPort](#) |
 27. Zoller, M.J. and Smith, M. 1983. Oligonucleotide-directed mutagenesis of DNA fragments cloned into M13 vectors. *Methods in Enzymol.* **100**: 468-500. | [Article](#) | [ISI](#) | [ChemPort](#) |
 28. Ogden, R.C. and Adams, D.A. 1987. Electrophoresis in agarose and acrylamide gels. *Methods in Enzymol.* **152**: 61-87. | [Article](#) | [ISI](#) | [ChemPort](#) |
 29. Hogan, B., Costantini, F. and Lacy, E. 1986. *Manipulating the Mouse Embryo: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 30. Schreier, M.H. and Tees, R. Long-term culture and cloning of specific helper T cells, p. 263-275. In: *Immunological Methods*. Vol. **2**. Academic Press, N.Y.

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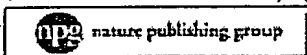
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1: Gene Ther. 2000 Jun;7(12):1046-54.

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**Effect of transgenic GDNF expression on gentamicin-induced cochlear and vestibular toxicity.**

Suzuki M, Yagi M, Brown JN, Miller AL, Miller JM, Raphael Y.

Kresge Hearing Research Institute, The University of Michigan, Ann Arbor 48109-0648, USA.

Gentamicin administration often results in cochlear and/or vestibular hair cell loss and hearing and balance impairment. It has been demonstrated that adenovirus-mediated overexpression of glial cell line-derived neurotrophic factor (GDNF) can protect cochlear hair cells against ototoxic injury. In this study, we evaluated the protective effects of adenovirus-mediated overexpression of GDNF against gentamicin ototoxicity. An adenovirus vector expressing the human GDNF gene (Ad.GDNF) was administered into the scala vestibuli as a rescue agent at the same time as gentamicin, or as a protective agent, 7 days before gentamicin administration. Animals in the Rescue group displayed hearing thresholds that were significantly better than those measured in the Gentamicin or Ad.LacZ/Gentamicin groups. In the Protection group, Ad.GDNF afforded significant preservation of utricular hair cells. The data demonstrated protection of the inner ear structure, and rescue of the inner ear structure and function against ototoxic insults. These experiments suggest that inner ear gene therapy may be developed as a clinical tool for protecting the ear against environmentally induced insults.

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Spiral Ganglion Neurons Are Protected from Degeneration by GDNF Gene Therapy

Masao Yagi, Sho Kanzaki, Kohei Kawamoto, Brian Shin, Pratik P. Shah, Ella Magal, Jackie Sheng, Yehoash Raphael

A¹ Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109-0506, USA

A² Department of Neuroscience, Amgen Inc., Thousand Oaks, CA 91320, USA

A³ Department of Otolaryngology, Kansai Medical University, Moriguchi, Osaka 570-8506, Japan

A⁴ Department of Otolaryngology, Keio University, Shinjuku-ku, Tokyo 160-0016, Japan

Abstract:

Perceptual benefits from the cochlear prosthesis are related to the quantity and quality of the patient's auditory nerve population. Multiple neurotrophic factors, such as glial cell line-derived neurotrophic factor (GDNF), have been shown to have important roles in the survival of inner ear auditory neurons, including protection of deafferented spiral ganglion cells (SGCs). In this study, GDNF gene therapy was tested for its ability to enhance survival of SGCs after aminoglycoside/diuretic-induced insult that eliminated the inner hair cells. The GDNF transgene was delivered by adenoviral vectors. Similar vectors with a reporter gene (lacZ) insert served as controls. Four or seven days after bilateral deafening, 5 ml of an adenoviral suspension (Ad-GDNF or Ad-lacZ) or an artificial perilymph was injected into the left scala tympani of guinea pigs. Animals were sacrificed 28 days after deafening and their inner ears prepared for SGC counts. Adenoviral-mediated GDNF transgene expression enhanced SGC survival in the left (viral-treated) deafened ears. This observation suggests that GDNF is one of the survival factors in the inner ear and may help maintain the auditory neurons after insult. Application of GDNF and other survival factors via gene therapy has great potential for inducing survival of auditory neurons following hair cell loss.

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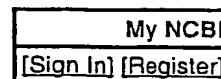
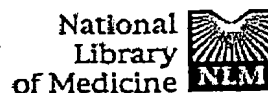
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FULL-TEXT ARTICLE

Introduction of the human growth hormone gene into the guinea pig mammary gland by in vivo transfection promotes sustained expression of human growth hormone in the milk throughout lactation.

Hens JR, Amstutz MD, Schanbacher FL, Mather IH.

Department of Animal and Avian Sciences, University of Maryland, College Park 20742, USA.

We tested the feasibility of transfecting mammary tissue in vivo with an expression plasmid encoding the human growth hormone (hGH) gene, under the control of the cytomegalovirus promoter. Guinea pig mammary glands were transfected with plasmid DNA infused through the nipple canal and expression was monitored in control and transfected glands by radioimmunoassay of milk samples for hGH. Sustained expression of hGH throughout lactation was attained with a polyion transfection complex shown to be optimal for the transfection of bovine mammary cells, in vitro. However, contrary to expectations, hGH expression was consistently 5- to 10-fold higher when DEAE-dextran was used alone for transfection. Thus polyion complexes which are optimal for the transfection of cells in vitro may not be optimal in vivo. The highest concentrations of hGH in milk were obtained when glands were transfected within 3 days before parturition. This method may have application for studying the biological role or physical properties of recombinant proteins expressed in low quantities, or for investigating the regulation of gene promoters without the need to construct viral vectors or produce transgenic animals.

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High-level synthesis of a heterologous milk protein in the mammary glands of transgenic swine

ROBERT J. WALL*, VERNON G. PURSEL*, AVI SHAMAY†, ROBERT A. MCKNIGHT†, CHRISTOPH W. PITTIUS†‡, AND LOTHAR HENNIGHAUSEN†§

*Reproduction Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705; and †Laboratory of Biochemistry and Metabolism, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892

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ABSTRACT The whey acidic protein (WAP) is a major milk protein in mice, rats, and rabbits but has not been found in milk of livestock including swine. To determine whether mammary gland regulatory elements from the WAP gene function across species boundaries and whether it is possible to qualitatively alter milk protein composition, we introduced the mouse WAP gene into the genome of swine. Three lines of transgenic swine were analyzed, and mouse WAP was detected in milk from all lactating females at concentrations of about 1 g/liter; these levels are similar to those found in mouse milk. Expression of the corresponding RNA was specific to the mammary gland. Our results suggest that the molecular basis of mammary-specific gene expression is conserved between swine and mouse. In addition the WAP gene must share, with other milk protein genes, elements that target gene expression to the mammary gland. Mouse WAP accounted for about 3% of the total milk proteins in transgenic pigs, thus demonstrating that it is possible to produce high levels of a foreign protein in milk of farm animals.

Milk protein genes are transcribed in the mammary gland of lactating animals, and the encoded proteins are secreted in large quantities into milk. The whey acidic protein (WAP) is an abundant milk protein in mice (1, 2) but has not been found in swine or other livestock. Expression of the WAP gene is confined to the mammary gland (2, 3) and is under the control of steroid and peptide hormones as well as other developmental signals during pregnancy (4–6).

By targeting synthesis of foreign proteins to the mammary gland of transgenic animals, it should be possible to produce valuable proteins on a large scale in milk (7, 8). The combined properties of high activity and tissue-specificity make the murine WAP gene promoter a good candidate for targeting gene expression to the mammary gland. Towards this end we previously have expressed a hybrid gene containing regulatory elements from the mouse WAP gene and coding sequences from human tissue plasminogen activator in the mammary gland of transgenic mice (5, 6) and analyzed the protein in milk (5, 9). By characterizing the WAP gene, it may be possible to use its control elements to target expression of hybrid genes in farm animals. However, it is not known whether mammary regulatory elements are gene specific and whether they are functional across species boundaries. In addition, it is not known if the presence of a novel protein may adversely affect the physiology of the mammary gland. To address these questions we introduced the unmodified mouse WAP gene (10) into swine, which themselves do not contain a WAP gene, and analyzed expression of RNA and protein. With this approach, potential problems in interpreting expression data from hybrid genes would not be a factor. Also, potential deleterious physiological effects of a foreign

protein might be minimized because the target gene encodes a milk protein that would be confined primarily to the mammary gland.

Swine were chosen for these studies because they offer both economy in animal resources and time when compared to ruminantia as a transgenic animal model and because the questions being addressed did not require harvesting large quantities of milk that would be more easily obtained from dairy animals such as cows, goats, or sheep. The two primary constraints in any large animal transgenic project are the number of fertilized ova obtainable and the number of embryo recipients available. On average it is possible to recover 2–3 times more injectable ova per donor gilt than can be collected from a cow, doe, or ewe. The efficiency of producing expressing transgenic pigs or sheep per injected ovum is about 0.3% (calculated from refs. 11 and 12). Though a live-born-expressing transgenic calf has not been reported, a larger number of ova will probably be required to produce an expressing transgenic cow (13). Furthermore, because swine are polytocous, a recipient sow can carry 5 times as many fetuses as a cow, doe, or ewe. Additionally, the generation interval of swine is ~11 months, whereas that of goats is between 11 and 21 months and that of cattle at least 24 months. Considering all of these factors, the use of swine rather than cows, goats, or sheep requires one-sixth the number of animals, with results obtainable in less than half the time.

MATERIALS AND METHODS

Production of Transgenic Pigs. Ovulation control and egg recovery were performed as described (14). Briefly, the time of ovulation of sexually mature gilts was controlled by feeding 15 mg of Altrenogest (R-2267, 17-allyl-hydroxyestra-4,9,11-trien-3-one, Roussel-Uclaf) daily for 5–9 days, beginning on day 12 and ending on day 15 of the estrous cycle. Twenty-four hours after the last feeding of Altrenogest, each gilt was given 1000 to 2000 international units of pregnant mare's serum gonadotropin (PMSG) by subcutaneous injection, and 79 hr later each gilt was given an intramuscular injection of 500 international units of human chorionic gonadotropin (hCG). Estrus behavior was monitored, and embryo donor gilts were either bred with a fertile boar or were artificially inseminated with fresh semen twice during estrus.

Approximately 58–61 hr after the hCG injection (18–21 hours after the expected time of ovulation), the reproductive tracts of donor gilts were exposed by midventral laparotomy during general anesthesia. Ova were recovered by flushing 20 ml of Dulbecco's phosphate-buffered saline (15) from the uterotubal junction through the cannulated infundibular end

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Abbreviation: WAP, whey acidic protein.

‡Present address: Hoechst AG, Frankfurt, Federal Republic of Germany.

§To whom reprint requests should be addressed.

of each oviduct. Recovered ova were immediately transferred into BMOC buffer (16) prior to microinjection and maintained at 38°C.

Pig ova are optically opaque and, as a consequence, their nuclear structures are not visible. However, centrifuging ova at $\approx 15,000 \times g$ for 3–8 min displaces the opaque material in the cytoplasm, thereby allowing the nuclear structures to be visualized (14). Pig ova were centrifuged, and a pronucleus of one-celled ova or both nuclei of two-celled ova were injected with a TE solution (1 mM Tris-HCl/0.1 mM EDTA, pH 7.2) containing ≈ 2 ng of a 7.2-kilobase (kb) *EcoRI* fragment per μ l that contained the mouse WAP gene (10). The fragment contained the entire transcribed region with its four exons, three introns, and 2.6-kb 5' and 1.6-kb 3' flanking sequences. Microinjections were performed with the aid of differential interference contrast optics at 200-fold magnification, essentially as described for mouse ova (17).

Between 20 and 30 injected ova were deposited into the ampullar region of one oviduct of each recipient gilt whose reproduction cycle had been synchronized with Altrenogest (but not superovulated—i.e., not given PMSG) or whose estrous cycle naturally coincided with the desired stage. Some recipients also received 2–4 uninjected control ova to increase the likelihood of maintaining pregnancy in the event that a majority of the microinjected eggs failed to develop. Time between microinjection and embryo transfer was about 30 min.

To identify transgenic piglets, DNA from tail biopsies was prepared and analyzed for the mouse WAP gene by Southern blotting. Offspring in the F_1 generation were analyzed by the polymerase chain reaction by using primers specific to the WAP gene.

Analysis of Mouse WAP. Milk whey proteins were separated under denaturing conditions in sodium dodecyl sulfate (SDS)/16% polyacrylamide gels and either stained with Coomassie Blue or transferred to nitrocellulose filters. After transfer the membrane was incubated overnight in TBS (20 mM Tris-HCl, pH 7.5/500 mM NaCl) containing 3% gelatin and then washed in TTBS (TBS containing 0.05% Tween 20). The membrane was then probed for 90 min with a 1:200 dilution of rabbit anti-WAP serum, followed by washing and incubation with alkaline phosphatase-conjugated goat anti-rabbit IgG in TBS containing 1% bovine serum albumin for 1 hr. The antibody-antigen complexes were stained with nitrobluetetrazolium and 5-bromo-4-chloro-3-indolyl phosphate in 100 mM Tris-HCl, pH 9.5/100 mM NaCl/5 mM $MgCl_2$.

Isolation of RNA and Northern Blot Analysis. During necropsy, tissues were immediately placed in liquid nitrogen and stored at -80°C , and total RNA was isolated (18). RNA samples containing 1 μ l of ethidium bromide solution (1 mg/ml) were electrophoresed in 1.5% agarose/formaldehyde gels. The gels were blotted onto GeneScreenPlus nylon membranes, which were then probed with a randomly primed labeled 450-base-pair (bp) cDNA fragment that spanned the mouse WAP coding region.

RESULTS

The Mouse WAP Gene in Transgenic Swine. Eight-hundred and fifty ova were recovered and microinjected, of which two-thirds were at the one-cell stage of development. The injected DNA contained 7.2 kb of the mouse WAP gene (see *Materials and Methods*, ref. 10). The microinjected ova along with 34 control ova were transferred into 29 recipient gilts. Twenty-two of the recipients carried their pregnancies to term, resulting in the birth of 189 pigs. DNA analysis of tail biopsies revealed that 5 (2 males and 3 females) of the piglets had incorporated the mouse WAP gene into their genomes. Approximately 1% of the injected ova resulted in transgenic

founders. From other transgenic pig projects using different gene constructs, the efficiency of producing founder pigs was similar (11). In this study one pig was stillborn and one died shortly after birth. Such deaths are not uncommon in the pig industry, where neonate mortality is in the range of 15–20%. Lines from the three surviving pigs were established, and offspring were analyzed. Male founder 1301 was bred to three nontransgenic females; 4 of 32 offspring were transgenic, suggesting that he was mosaic for the WAP gene. Transgenic mouse breeding studies have estimated that about 30% of transgenic founders are germ-line mosaics (19). Based on Southern blot analyses, this line contains ≈ 10 intact copies of the WAP gene in a head-to-tail arrangement at a single locus. Female founder 2202 carried ≈ 15 copies of the WAP gene. She was bred at 8 months of age; 4 of 9 offspring were transgenic. She was bred a second time and died of an unknown cause 4 days before anticipated parturition. The two transgenic daughters from her first litter were also bred, and after farrowing, milk and RNA were analyzed. Female founder 1302, carrying ≈ 10 copies of the WAP gene, was unsuccessfully bred three times. After the third failure, she was superovulated as a means of diagnosing the cause of her reproductive failures and to collect eggs if the cause did not involve ovarian disfunction. Twenty-eight ova were recovered and transferred to two recipients. From these, 20 piglets were born of which 8 were transgenic. Apparently not all of female founder 1302's eggs had been recovered because she subsequently gave birth to 9 piglets, 5 of which were transgenic.

Secretion of Mouse WAP into Pig Milk. Expression of the WAP transgene in transgenic pigs was evaluated by both protein and RNA analyses. Milk from female founder 2202 and her daughter 5403, from two daughters (5511 and 5701) of male founder 1301, and from female founder 1302, was analyzed for the presence of mouse WAP. Milk proteins were separated in SDS/polyacrylamide gels and either stained with Coomassie blue or blotted onto nitrocellulose membranes and analyzed with anti-mouse WAP antibodies. WAP has a molecular mass of about 14 kDa (Fig. 1A, lane 8) and, at a concentration of about 2 mg per ml, constitutes the major

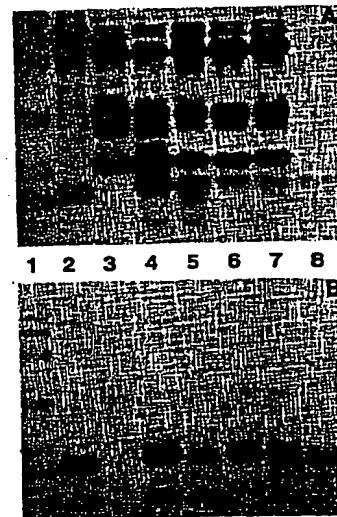


FIG. 1. Secretion of mouse WAP into milk of transgenic pigs. Milk proteins (20 μ g) were separated in SDS/polyacrylamide gels and either stained (A) or analyzed with rabbit anti-WAP antibodies (B). Lanes: 1, molecular mass markers (14, 18, 29, 45, 68, and 96 kDa); 2, total mouse whey proteins; 3–7, milk from nontransgenic pig (lane 3), pig 2202 (lane 4), pig 5403 (lane 5), pig 5701 (lane 6), and pig 5511 (lane 7); 8, 1 μ g of purified mouse WAP.

wey protein in mice (Fig. 1A, lane 2). A protein comigrating with mouse WAP was present in the milk of transgenic pigs (Fig. 1A, lanes 4–7) but not in milk from a nontransgenic control pig (Fig. 1A, lane 3). In addition, a 14-kDa protein in milk from transgenic, but not from nontransgenic, pigs reacted strongly with anti-mouse WAP antibodies (Fig. 1B). The lower molecular mass material reacting with anti-WAP antibodies probably reflects degradation products of the WAP. Taken together, this shows that the mouse WAP gene was expressed in transgenic pigs, and the encoded protein was secreted into the milk. The level of mouse WAP in the milk of each transgenic pig was determined in ELISA. By setting the level of WAP in mouse milk arbitrarily at 100%, animals 2202 and 5403 (line 2202) and animals 5701 and 5711 (line 1301) were shown to express WAP at about 100%, and female founder 1302, at about 50%. Thus, about 1–2 g of WAP was present per liter of pig milk.

WAP is secreted into mouse milk during the entire lactational period. To determine whether the expression in transgenic pigs paralleled this pattern, we analyzed WAP levels in the milk of founder female 1302 over a 4-week lactational period (Fig. 2). Whey samples were separated in SDS/polyacrylamide gels and either stained (Fig. 2A) or analyzed with anti-WAP antibodies (Fig. 2B). Constant levels of WAP were found over a 26-day period. This suggests that, at least over this period of time, the WAP transgene was coordinately regulated with other pig milk protein genes.

Expression of Mouse WAP RNA in Pigs. To correlate the level of WAP in milk with the corresponding RNA in mammary tissue, founder female 2202 was biopsied 11 days postpartum, and mammary RNA was analyzed with a mouse-specific WAP cDNA. An RNA of about 600 nucleotides hybridized with the WAP probe (Fig. 3, lanes b and c), confirming mouse WAP gene expression in the mammary glands of transgenic pigs. Furthermore, the RNA levels in pig 2202 and mouse were similar; this agrees with the WAP levels found in the milk. The WAP RNA in pig 2202 appeared to be

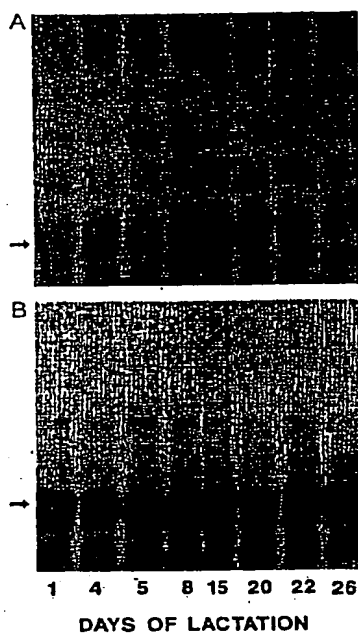


FIG. 2. Expression of mouse WAP during the lactational period of pig 1302. Milk samples were collected at various days after parturition as indicated, and whey fractions were prepared. Upon gel separation, samples were either stained (A) or analyzed with anti-WAP antibodies (B).

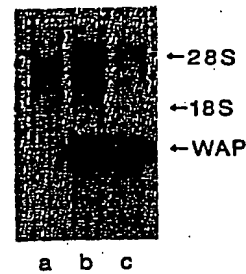


FIG. 3. Expression of mouse WAP RNA in transgenic pigs. Mammary RNAs (5 μ g) from a lactating nontransgenic pig (lane a), founder pig 2202 (lane b), and a mouse (lane c) were separated in a formaldehyde gel, transferred to a nylon membrane, and analyzed with a cloned cDNA probe specific for mouse WAP RNA.

about 10–20 nucleotides shorter than its counterpart in mice (Fig. 3). Since the protein coding region was intact, the smaller size may be due to differences in polyadenylation. RNA from a nontransgenic pig did not hybridize with the WAP probe (Fig. 3, lane a), verifying the absence of an endogenous WAP RNA in the pig mammary gland.

In lactating mice the WAP gene is expressed almost exclusively in the mammary gland with levels in nonmammary tissues at least 4 orders of magnitude lower (5). To test whether the 7.2-kb WAP transgene contained elements for stringent tissue specificity observed in mice, we analyzed tissues from lactating pigs from lines 2202 and 1301 for the presence of WAP RNA (Fig. 4). To demonstrate potential WAP expression in nonmammary tissues, we exposed the RNA blot for 24 hr (Fig. 4a and c). The specificity of WAP hybridization and the quantity of WAP RNA in the mammary gland were assessed in a 30-min exposure (Fig. 4b). In animal 5701 (line 1301), WAP RNA was only found in the mammary gland (Fig. 4c) at a level similar to that seen in a 10-day lactating mouse. The sensitivity of the assay would have

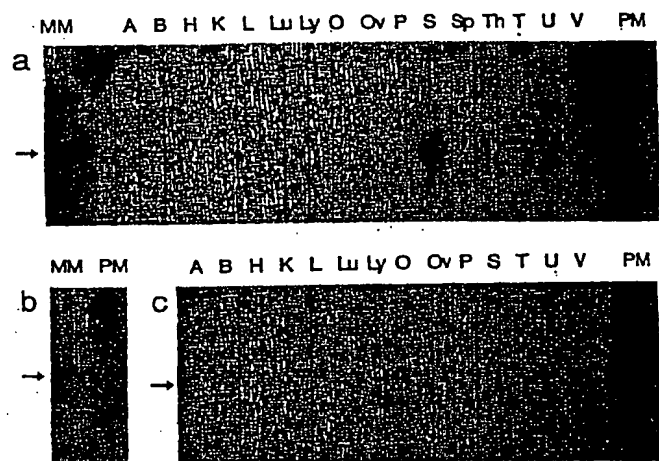


FIG. 4. Tissue distribution of WAP RNA in transgenic pigs. Pigs 5403 (a) and 5701 (c) were sacrificed, and RNA was prepared from several tissues. Upon separation in formaldehyde gels and transfer to nylon membranes, the RNA was analyzed with a probe specific for mouse WAP RNA. Lanes: MM, mouse mammary gland; PM, pig mammary gland; A, adrenals; B, brain; H, heart; K, kidney; L, liver; Lu, lung; Ly, lymph node; O, ovaries; Ov, oviduct; P, pituitary; S, salivary gland; Sp, spleen; Th, thymus; T, tongue; U, uterus; V, vulva. In a and c, 20 μ g of total RNA was loaded in lanes with the exception of mouse mammary gland (lane MM), where 4 μ g was loaded. (b) One-hour exposure of the MM and PM lanes of a. Arrows indicate the position of WAP RNA.

permitted detection of WAP RNA levels 1000-fold lower than that observed. The level of WAP RNA in animal 5403 (line 2202) was about 80% of that seen in mouse (Fig. 4a). The lower molecular mass band in the vulva RNA from animal 5701 was not reproducible and probably reflects a gel or blotting artifact. In animal 5403 WAP expression was detected in salivary gland, although at a level of only 1% of that seen in mammary tissue (Fig. 4a). Low-level expression in the salivary gland also has been described for other transgenes containing regulatory elements from milk protein genes (5, 20). Although the salivary gland and mammary gland have similar developmental patterns in that they require interaction between epithelial and mesenchymal tissue for proper duct formation to occur (21, 22), they are not considered closely related. In contrast, sebaceous glands have a common developmental origin to that of the mammary gland. However, no WAP transcripts were found in tissue taken from the vulva (Fig. 4), which is rich in sebaceous glands.

DISCUSSION

Three lines of transgenic swine containing the mouse WAP gene have been generated and analyzed. Although swine does not contain an endogenous WAP gene, its transcription machinery recognized the mouse WAP transgene in a tissue-specific manner, and mouse WAP was secreted into milk from founder swine as well as their offspring at levels similar to those seen in mouse milk. Thus, the molecular basis for mammary-specific gene expression is conserved between swine and mouse, and it can be suggested that the mouse WAP gene shares mammary regulatory elements with pig milk protein genes.

Expression levels of the mouse WAP genes in three lines of transgenic pigs described here and in three additional lines (unpublished data), which carry between 10 and 20 copies of the transgene, were consistently high and at a level comparable to the expression level of the endogenous gene in mice. Activity of the WAP gene in pigs appears to be relatively independent of the site of integration into host chromosomes and also independent of the gene copy number. In contrast, expression of the same 7.2-kb mouse WAP gene in transgenic mice was highly dependent on the integration site of the transgene (36). It remains to be determined whether the consistently high-level expression in transgenic pigs reflects special properties of the WAP gene, such as the presence of dominant transcription elements, or whether the pig genome provides a unique permissive environment for transgene expression. A host of other transgenic swine projects (23) argues against the latter explanation. Data from the sheep β -lactoglobulin gene (24), the rat WAP (25) and β -casein (26) genes, and several hybrid genes containing mammary regulatory elements (27–30) have shown that expression was influenced by the site of integration in transgenic mice. At a minimum the present study suggests that WAP gene regulation is different in mice and swine.

This study shows that it is feasible to synthesize and secrete a heterologous milk protein in the milk of farm animals at relatively high concentrations—i.e., more than 1 g/liter. Clark and colleagues had shown that hybrid genes containing regulatory elements from the sheep β -lactoglobulin gene are expressed in the mammary glands of transgenic sheep (31). However, the concentrations of the encoded proteins factor IX and α_1 -antitrypsin were only 25 μ g/liter and 5 mg/liter, respectively (31). With another transgene, this group produced human α_1 -antitrypsin in mouse milk at levels of more than 1 g/liter (20). Therefore, the ability of a transgene to be expressed in the mammary gland at high levels does not appear to be related to the nature of the encoded protein (milk protein versus foreign protein) but rather to the presence of appropriate transcription elements.

We are currently testing the ability of the mouse WAP gene promoter to control expression of non-WAP structural gene sequences in pigs.

The concentration of the transgene product produced in this study should be encouraging to those who envision using the mammary gland as a bioreactor for the production of foreign proteins as an economically viable alternative to existing tissue and microbial culture systems (7, 8). Swine produce about 10 kg of milk per day (32), and, based on the expression levels discussed here, it should be possible to produce the protein of interest at a rate of about 1 kg per lactational period of 7 weeks. Since the WAP gene promoter is active in pigs during their entire lactational period, this appears to be an achievable goal, and one sow could satisfy current world's demand of blood clotting factor IX. Alternatively, to the dairy industry, the modification of the composition of milk proteins themselves may be desirable so that overexpressing heterologous or endogenous milk proteins would result in novel milk products (33).

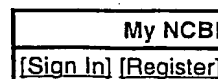
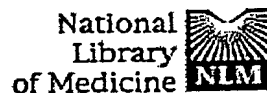
As with other expression systems, high activity of the transgene could have adverse effects on the physiology of the mammary gland. Pigs from two lines (1301 and 2202) were unable to sustain lactation. In contrast, lactation persisted normally in female founder 1302. This animal secreted less WAP into milk than those that abrogated lactation. Agalactia has not been observed in transgenic mice that secrete into their milk heterologous milk proteins (24, 34) or pharmacologically active proteins (20, 35) at levels similar to or exceeding those described here with swine. Experiments are in progress to determine whether the premature termination of lactation exhibited by some of the pigs is associated with mammary gene expression.

Note Added in Proof. We have generated transgenic mice with the 7.2-kb WAP transgene described in this paper and observed that some of the animals cannot maintain lactation (T. Burdon, R.J.W., and L.H., unpublished data).

We thank Floyd Schanbacher for purified mouse WAP, Leah Schulman and Mark Spencer for technical assistance, Jim Platt for animal care, and William Jakoby for continued support.

1. Piletz, J. E., Heinlen, M. & Ganshow, R. E. (1981) *J. Biol. Chem.* 256, 11509–11516.
2. Hennighausen, L. G. & Sippel, A. E. (1982) *Eur. J. Biochem.* 125, 131–141.
3. Hennighausen, L. G. & Sippel, A. E. (1982) *Nucleic Acids Res.* 10, 2677–2684.
4. Hobbs, A. A., Richards, D. A., Kessler, D. J. & Rosen, J. M. (1982) *J. Biol. Chem.* 257, 3598–3605.
5. Pittius, C. W., Hennighausen, L., Lee, E., Westphal, H., Nichols, E., Vitale, J. & Gordon, K. (1988) *Proc. Natl. Acad. Sci. USA* 85, 5874–5878.
6. Pittius, C. W., Sankaran, S., Topper, Y. & Hennighausen, L. (1988) *Mol. Endocrinol.* 2, 1027–1032.
7. Clark, A. J., Simons, P., Wilmut, I. & Lathe, R. (1987) *Tibtech* 5, 20–24.
8. Hennighausen, L., Ruiz, L. & Wall, R. J. (1990) *Curr. Opin. Biotech.* 1, 74–78.
9. Gordon, K., Lee, E., Vitale, J. A., Smith, A. E., Westphal, H. & Hennighausen, L. (1987) *Bio/Technology* 5, 1183–1187.
10. Campbell, S. M., Rosen, J. M., Hennighausen, L., Strech-Jurk, U. & Sippel, A. E. (1984) *Nucleic Acids Res.* 12, 8685–8697.
11. Pursel, V. G., Hammer, R. E., Bolt, D. J., Palmiter, R. D. & Brinster, R. L. (1990) *J. Reprod. Fertil. Suppl.* 41, 77–87.
12. Rexroad, C. E., Hammer, R. E., Behringer, R. R., Palmiter, R. D. & Brinster, R. L. (1990) *J. Reprod. Fertil. Suppl.* 41, 119–124.
13. Massey, J. M. (1990) *J. Reprod. Fertil. Suppl.* 41, 199–208.
14. Wall, R. J., Pursel, V. G., Hammer, R. E. & Brinster, R. L. (1985) *Biol. Reprod.* 32, 645–651.
15. Dulbecco, R. & Vogt, M. (1954) *J. Exp. Med.* 99, 167–175.

16. Brinster, R. L. (1972) in *Growth, Nutrition, and Metabolism of Cells in Culture*, eds. Rothblatt, G. & Cristofalo, V. (Academic, New York), Vol. 2, pp. 251-286.
17. Gordon, J. W., Scangos, G. A., Plotkin, D. J., Barbosa, J. A. & Ruddle, F. H. (1980) *Proc. Natl. Acad. Sci. USA* 77, 7380-7384.
18. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* 162, 156-159.
19. Wilkie, T. M., Brinster, R. L. & Palmiter, R. D. (1986) *Dev. Biol.* 118, 9-18.
20. Archibald, A. L., McClenaghan, M., Hornsey, V., Simons, J. P. & Clark, A. J. (1990) *Proc. Natl. Acad. Sci. USA* 87, 5178-5182.
21. Mepham, T. B. (1987) in *Physiology of Lactation* (Open Univ. Press, Philadelphia), pp. 1-14.
22. Hopper, A. F. & Hart, N. H. (1985) in *Foundations of Animal Development* (Oxford Univ. Press, New York), 2nd Ed., pp. 302-337.
23. Pursel, V. G., Pinkert, C. A., Miller, K. F., Bolt, D. J., Campbell, R. G., Palmiter, R. D., Brinster, R. L. & Hammer, R. E. (1989) *Science* 244, 1281-1288.
24. Simons, J. P., McClenaghan, M. & Clark, A. J. (1987) *Nature (London)* 328, 530-532.
25. Bayna, E. M. & Rosen, J. M. (1990) *Nucleic Acids Res.* 18, 2977-2985.
26. Lee, K.-F., DeMayo, F. J., Atiee, S. H. & Rosen, J. M. (1988) *Nucleic Acids Res.* 16, 1027-1041.
27. Shu-Hua, Y., Deen, K. C., Lee, E., Hennighausen, L., Sweet, R. W., Rosenberg, M. & Westphal, H. (1989) *Mol. Biol. Med.* 6, 255-261.
28. Andres, A.-C., Schönenberger, C.-A., Groner, B., Hennighausen, L., LeMeur, M. & Gerlinger, P. (1987) *Proc. Natl. Acad. Sci. USA* 84, 1299-1303.
29. Lee, K.-F., Atiee, S. H. & Rosen, J. M. (1989) *Mol. Cell. Biol.* 9, 560-565.
30. Bühler, T. A., Bruyere, T., Went, D. F., Stranzinger, G. & Bürki, K. (1990) *Bio/Technology* 8, 140-146.
31. Clark, A. J., Bessos, H., Bishop, J. O., Brown, P., Harris, S., Lathe, R., McClenaghan, M., Prowse, C., Simons, J. P., Whitelaw, C. B. A. & Wilmut, I. (1989) *Bio/Technology* 7, 487-492.
32. Harkins, M., Boyd, R. D. & Bauman, D. (1989) *J. Anim. Sci.* 67, 1997-2008.
33. Hennighausen, L. (1990) *Protein Express. Purif.* 1, 3-8.
34. Vilotte, J.-L., Soulier, S., Sinnakre, M.-G., Massoud, M. & Mercier, J.-C. (1989) *Eur. J. Biochem.* 186, 43-48.
35. Meade, H., Gates, L., Lacy, E. & Lonberg, N. (1990) *Bio/Technology* 8, 443-446.
36. Burdon, T., Wall, R. J., Sankaran, L. & Hennighausen, L. (1991) *J. Biol. Chem.*, in press.



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Transgenic chickens: insertion of retroviral genes into the chicken germ line.

Salter DW, Smith EJ, Hughes SH, Wright SE, Crittenden LB.

We infected early chicken embryos by injection of wild-type and recombinant avian leukosis viruses into the yolk of unincubated, fertile eggs. The viremic males (designated generation 0 (G-0) were tested for transmission of proviral DNA to their G-1 progeny. Nine of 37 G-0 viremic males were mosaic and proviral DNA was transmitted to their progeny at frequencies varying from 1 to 11%. All of the G-1 progeny examined by restriction enzyme analysis for clonality of proviral junction fragments had one to three simple but different fragments. The proviral DNA was transmitted from G-1 to the G-2 progeny in a Mendelian fashion thus proving that retroviral genes have been inserted into the chicken germ line. One of the viruses is a candidate vector for insertion of foreign genes into the chicken germ line.

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Replication-Defective Vectors of Reticuloendotheliosis Virus Transduce Exogenous Genes into Somatic Stem Cells of the Unincubated Chicken Embryo

ROBERT A. BOSSELMAN,^{1*} ROU-YIN HSU,¹ TINA BOGGS,¹ SYLVIA HU,¹ JOAN BRUSZEWSKI,¹ SUSAN OU,^{1†} LARRY SOUZA,¹ LEE KOZAR,¹ FRANK MARTIN,¹ MARGERY NICOLSON,¹ WILLIAM RISHELL,² JOSEPH A. SCHULTZ,² KENNETH M. SEMON,² AND R. GREGORY STEWART^{2‡}

Amgen Inc., 1900 Oak Terrace Lane, Thousand Oaks, California 91320,¹ and Arbor Acres Farm, Inc., Marlborough Road, Glastonbury, Connecticut 06033²

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Replication-defective vectors derived from reticuloendotheliosis virus were used to transduce exogenous genes into early somatic stem cells of the chicken embryo. One of these vectors transduced and expressed the chicken growth hormone coding sequence. The helper cell line, C3, was used to generate stocks of vector containing about 10^4 transducing units per ml. Injection of 5- to 20- μ l volumes of vector directly beneath the blastoderm of unincubated chicken embryos led to infection of somatic stem cells. Infected embryos and adults contained unrearranged integrated proviral DNAs. Embryos expressed the transduced chicken growth hormone gene and contained high levels of serum growth hormone. Blood, brain, muscle, testis, and semen contained from individuals injected as embryos contained vector DNA. Replication-defective vectors of the reticuloendotheliosis virus transduced exogenous genes into chicken embryonic stem cells in vivo.

Insertion of genetic information into the chicken provides a new in vivo approach to analyzing gene expression and its effects on avian physiology. A vector derived from Rous sarcoma virus has been used to transfer additional growth hormone genes into chicken somatic cells by infection of 7- and 9-day-old embryos (35). More recently, gene transfer into chicken germ cells (27-29) has been accomplished by infection of day-old embryos with similar replicating Rous sarcoma virus vectors (18, 33). This approach to avian gene transfer has advantages over DNA microinjection since the early chicken zygote is difficult to manipulate and even a freshly laid egg contains thousands of cells (10, 20). However, replicating retroviral vectors have disadvantages. They can result in gene transfer to susceptible cells at various stages of differentiation long after initial infection of the embryo. This can make it difficult to determine the stage of development at which gene insertion takes place or the cell lineage relationships within fully differentiated tissues. Furthermore, replicating vectors also increase the potential for disease states associated with chronic viral infection (16, 24, 38).

Replication-defective retroviral vectors offer an alternative approach (2, 6, 21, 36, 40). Such vectors, derived from reticuloendotheliosis virus type A (REV-A) (31), are produced by the helper cell line C3 which contains a packaging-defective helper provirus (40). When transfected with a defective proviral vector, this helper cell assembles infectious replication-defective vector but little or no competent virus (17). Both replicating REV and the replication-defective REV vector ME111 have been previously used for gene transfer into chicken somatic cells by injection of virus into follicles before ovulation (32). We have used a method of

gene transfer based on microinjection of vector into early embryos.

This report describes the transfer of new genetic information, including additional chicken growth hormone (cGH) coding sequences, into somatic stem cells of the chicken embryo. Chickens do not generally contain endogenous REV and express endogenous cGH only in the pituitary during late embryogenesis and after hatching (15, 19, 30). In vivo, these vectors can infect somatic stem cells of day-old chicken embryos, resulting in precociously high levels of circulating cGH and the presence of vector DNA in a variety of adult somatic tissues.

MATERIALS AND METHODS

Cells. The REV-A helper cell line, C3, was generously provided by H. Temin (40). C3 cells were cultured in minimal essential medium (Eagle) containing 7% fetal calf serum—400 μ g of G418 per ml. Chicken embryo fibroblasts (CEF) were grown in F-10 medium supplemented with 10% tryptose phosphate broth—5% calf serum. D17 cells were cultured in minimum essential medium (Eagle)—7% fetal calf serum (40). Buffalo rat liver thymidine kinase (TK)-negative (BRLtk⁻) cells were grown in minimum essential medium (Eagle) plus 7% calf serum (39). QT-6 cells were obtained from C. Moscovici and grown as described previously (23).

Virus infection. BRLtk⁻ cells were infected in medium containing 100 μ g of Polybrene per ml. CEF were infected in normal medium. Cells were usually exposed to virus overnight.

Vectors. The ME111 vector has been previously described (8). The vector SW272/cGH was derived by insertion of cGH cDNA downstream of the 5' long terminal repeat (LTR) of the SW272 vector (39).

Vector assays. TK transducing units (TKTU) released by 5×10^5 C3 helper cells stably transfected with vector SW272/cGH were harvested after 6 h of incubation and were assayed by infection of 10^5 BRLtk⁻ cells. TK-positive cells were selected for growth in medium (40) containing 1×10^{-4}

* Corresponding author.

† Present address: Department of Biology, California Institute of Technology, Pasadena, CA 91125.

‡ Present address: Poultry Research Center, University of Georgia, Athens, GA 30601.

M hypoxanthine, 3×10^{-6} M thymidine, and 5×10^{-7} M methotrexate.

Plasmids and chicken DNAs. All plasmid DNAs were propagated by using derivatives of pBR322 and the HB101 strain of *Escherichia coli*. The plasmid pSW272 contains a derivative of the spleen necrosis provirus (SNV), lacks most sequences encoding the structural genes of the virus, and contains the herpes simplex virus type 1 (HSV-1) *tk* gene (40). Chicken genomic DNAs were isolated from Arbor Acres males of meat breeding lines.

Nucleic acid isolation. Chicken embryo DNA was prepared by solubilizing tissue in buffer containing 100 mM EDTA, 1% sodium dodecyl sulfate, 100 μ g of proteinase K per ml (pH 8). Samples were incubated at 60°C for 15 min, then at 37°C with additional protease (100 μ g/ml) for 4 h. The DNA was sheared, adjusted to 200 mM NaCl, and extracted twice with equal volumes of phenol and chloroform-isoamyl alcohol (24:1) and once with 2 volumes of chloroform-isoamyl alcohol. DNA was ethanol precipitated and dissolved in 0.01 M Tris-0.001 M EDTA (pH 8.0). Unsheared DNA was used for Southern blot analysis (34).

Nucleic acid analysis. DNA samples were applied to Gene Screen Plus membranes (New England Nuclear Co.) for dot blot analysis by means of 96-well plexiglass manifolds. DNA on membranes was denatured in 1.5 M NaCl-0.5 M NaOH for 15 min, neutralized in 0.5 M Tris(pH 7.5)-1.5 M NaCl for 1 min, blotted dry, and baked at 80°C for 30 min. Hybridizations were carried out as already described (17). Radiolabeled DNA probe was prepared by the method of random priming (13). Southern blot analysis was performed as described previously (34).

cGH analysis. cGH expression was analyzed either by radioimmunoassay (RIA) (35) or by Western immunoblotting (3).

Transfection. The REV-derived helper cell line, C3, was transfected as previously described (14) with the plasmids pSW272/cGH and pHyG (37). Transfected cells were selected for 10 to 14 days in medium containing 200 μ g of hygromycin per ml.

Embryo infection. Shell was removed from the area above the blastoderm of unincubated eggs. A Narishige micromanipulator and a 25- μ l Drummond pipette fitted with a glass needle were used to inject 5- to 20- μ l volumes of cell culture medium containing vector directly beneath the exposed blastoderm. The titer of vector was about 10^4 TKTU/ml as measured on BRLtk⁻ cells. The relative titer of this vector on chicken embryo cells in vivo is unknown. Eggs were resealed with a patch of shell membrane which was covered with Devcon Duco cement and allowed to dry. Eggs were incubated at 37.8°C.

RESULTS

Vectors ME111 and SW272/cGH. The sequence relationships among SNV, ME111, the cGH transducing vector SW272/cGH, and the packaging-defective helper proviruses present in C3 helper cells are shown in Fig. 1. ME111 has been described in detail elsewhere (8). The parental vector SW272 is derived from SNV and contains the HSV-1 *tk* gene and promoter in the same transcriptional orientation as the viral promoter (39). The cGH coding sequence was originally derived from a cDNA clone made from chicken pituitary mRNA (35). A DNA fragment *Xba*I to *Nco*I contains the complete coding sequence of the cGH gene but lacks the poly(A) addition signal present at the 3' end of the cDNA. Using Klenow reagent and blunt-end ligation, the cGH sequences were inserted into the unique *Xba*I site within

pSW272 located just downstream of the viral 5' splice donor and packaging sequence, 555 nucleotides from the 5' end of the viral RNA transcript (39). The orientation of the cGH coding sequence is the same as that of the viral sequences. Proceeding from the 5' end of the proviral RNA transcript of SW272/cGH, the first ATG encountered codes for the N-terminal methionine of cGH. SW272/cGH is designed to express cGH mRNA transcripts from the viral promoter.

Transduction and expression of REV vectors in vitro. Careful screening of the C3 helper cells transfected with pSW272/cGH and pHyg yielded clone C3-44 which released 2×10^4 TKTU/ml into growth medium but very low levels of competent virus. Competent REV in these cultures, as estimated by infection of cultured CEF, was about 10 infectious units of REV per ml or less (17). Western blot analysis of cGH released by C3-44 cells revealed a predominantly single band of protein which comigrated with purified recombinant cGH (Fig. 2). The observed molecular size of cGH was about 23,000 daltons. The estimated concentration of cGH in a 72-h harvest of medium of clone C3-44 was at least 500 ng/ml (data not shown). CEF infected with vector released >40 ng of cGH per ml of growth medium as determined by RIA 3 days after infection (data not shown). Western blot analyses of cGH released by cell lines infected with the SW272/cGH vector are shown in Fig. 2, lanes 13 through 18. Cell lines B56 and B20 derive from the canine cell line D17. Cell lines QT82, QT54, QT15, and QT8 derive from the quail cell line QT-6. All of these cells release cGH having the same apparent molecular size as purified recombinant cGH (23 kilodaltons) (35). Approximate levels of cGH expression varied from 2 to 10 ng/ml.

Analysis of DNA from chicken embryos after vector infection. Tissue culture fluid (20- μ l volumes) containing the vector SW272/cGH was injected beneath the blastoderms of unincubated chicken embryos. Total embryonic DNA was isolated from vector-injected and uninjected control embryos after 7 days of development and was analyzed by qualitative dot blot hybridization with either a radiolabeled cGH probe (Fig. 3A) or a REV vector probe (Fig. 3B). The cGH probe was used to demonstrate that sufficient DNA was present on the filter for detection of vector sequences present at low copy number. Of 25 injected embryos, 13 (52%) hybridized to a radiolabeled probe of vector DNA, whereas control DNA from uninjected embryos did not.

To confirm the presence and correct genome organization of vector sequences in infected 7-day embryos, high-molecular-size DNAs from 10 vector-containing embryos were digested with *Bam*HI endonuclease and subjected to Southern blot analysis (34) (Fig. 4). The embryo DNAs examined included those from Fig. 3B, rows 1a, 2a, 6a, 7a, and 8a. Internal *Bam*HI fragments predicted from the cGH vector sequence are diagrammed in Fig. 1. Digestion of integrated proviral vector sequences of SW272/cGH should yield DNA fragments internal to the provirus of 0.86, 2.3, and 1.6 kilobase pairs (kb). A 5' junction fragment containing the 5' LTR of the vector linked to host cellular sequences adjacent to the integration site might also be detected. No 3' junction fragment containing host DNA sequences would be detected, because a *Bam*HI restriction endonuclease site is located at the 3' end of the proviral LTR. As shown in Fig. 4A, lanes 3 to 7 and 12 to 16, DNAs from these vector-infected embryos show the expected *Bam*HI DNA fragments of 0.86, 2.3, and 1.6 kb when analyzed with a probe derived from the complete SW272 plasmid DNA, which does not contain cGH sequences. The absence of detectable *Bam*HI fragments containing the junction of cellular DNA and integrated vector DNA indicates multiple sites of vector

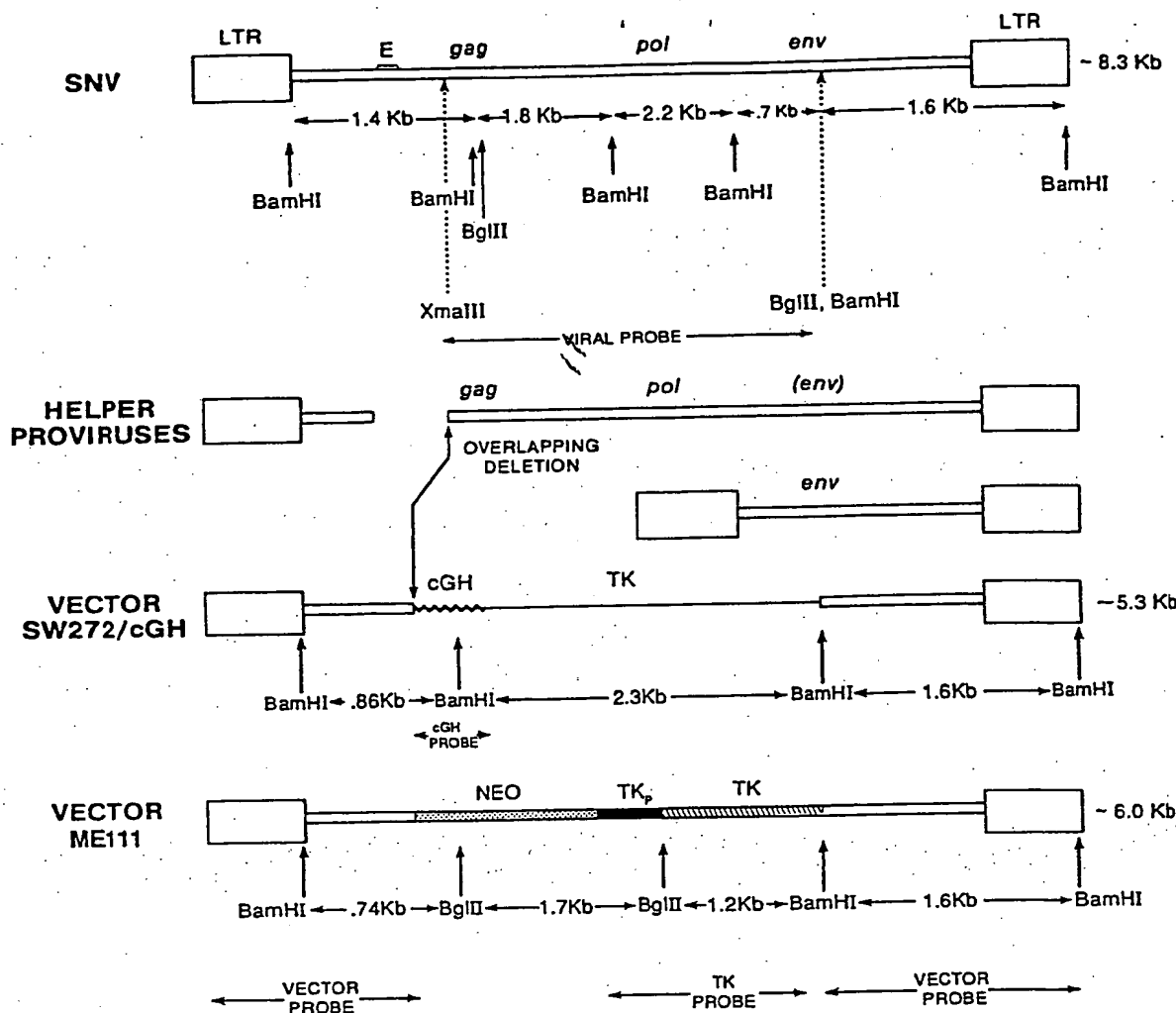


FIG. 1. Sequence relationships among the parental SNV provirus, the modified packaging-defective helper proviruses, and the vectors ME111 and SW272/cGH are shown. Relevant features of these proviruses include the LTRs, the structural genes of the virus (*gag*, *pol*, *env*), the approximate position of the packaging sequence (E), the *cGH* sequences, the HSV-1 *tk* gene promoter (TKp), the *tk* coding sequence (TK), and the neomycin phosphotransferase coding sequence (NEO). The (*env*) sequence in the larger of the two helper proviruses is presumably not expressed because of removal of the 5' splice donor. Overlapping deletions indicated between helper and vector sequences should reduce recombination between these genomes. A description of the REV helper proviruses and the original TK transducing vector pSW272 and ME111 have been given (8, 40). The 5' LTRs of both helper proviruses derive from SNV. Their coding sequences derive from REV-A. The *env* helper provirus lacks viral splice donor and acceptor sequences. The first ATG is that of the *env* gene. The cGH vector derives from SNV. REV-A and SNV share high sequence homology. Relative sizes (in kilobases) of *Bam*HI restriction endonuclease fragments are indicated. Also given are the locations of viral, vector, TK, and cGH DNA probes.

provirus integration during infection of early embryonic cells. No 0.57-kb *Bam*HI fragment predicted from the structure of unintegrated circular forms of either the vector DNA or helper virus DNA was observed. No 1.4-kb fragment diagnostic of the 5' end of integrated replication-competent proviral SNV DNA was observed (39) (see Fig. 1). *Bam*HI-digested DNA from uninjected whole embryos or from blood of uninjected chickens did not hybridize to the vector probe (Fig. 4A, lanes 2, 8, 11, and 17, respectively).

After removal of the SW272 probe (Fig. 4B), the same filters were hybridized with a viral probe specific for the structural genes of REV to detect the presence of replication-competent virus (Fig. 4C). The parental SNV and REV-A proviruses used to derive the helper cell and vectors described here contain internal *Bam*HI fragments of 1.4, 1.8,

2.2, 0.7, and 1.6 kb (see Fig. 1). Only the 1.6-kb fragment would not be detected by the virus-specific probe (Fig. 1) used in this analysis. No virus-specific *Bam*HI fragments were observed, indicating that endogenous and exogenous REV sequences were not detectable (Fig. 4C). Although this result does not rule out the presence of competent helper virus, it shows that efficient gene transfer takes place via the replication-defective SW272/cGH vector. The dot blot on the right of panel C contains various quantities of plasmid pSW253 which carries the entire REV provirus (5).

The filters shown in Fig. 4C were washed to remove probe (Fig. 4D) and were reanalyzed with a cGH-specific probe (Fig. 4E). The fragments of 0.86 and 2.3 kb in lanes 3 to 7 and 12 to 16 are the predicted cGH-containing vector sequences described in Fig. 1. The two bands (asterisks) of approxi-

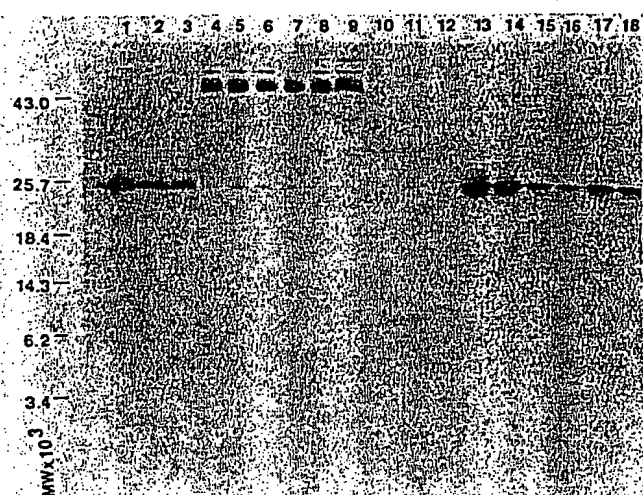


FIG. 2. Western blot analysis of cGH. Lanes: 1 to 3, 250, 125, and 30 ng per lane, respectively, of purified recombinant cGH; 4, 10 μ l of conditioned QT-6 medium; 5 to 9, immunoprecipitated cGH from various volumes of serum from 15-day vector-injected embryos, birds 18 (80 μ l), 2 (100 μ l), 6 (60 μ l), 30 (100 μ l), and 27 (200 μ l), respectively (see Table 1); 10 to 12, sample buffer alone; 13 to 18, 10 μ l of conditioned medium from clones of SW272/cGH-infected D17 and QT-6 cells (clones B56, B20, QT82, QT54, QT15, and QT8, respectively). Molecular weights (MW $\times 10^3$) are shown at the left.

mately 6.4 kb and approximately 2.7 kb, which are common to all lanes, represent *Bam*HI fragments derived from the endogenous cGH gene. As expected, embryo DNAs in lanes 3 to 7 and 12 to 16 contain all four fragments derived from both the vector and endogenous gene. The 1.6-kb *Bam*HI fragment present in lanes 3 to 7 and 12 to 16 of Fig. 4A is missing in Fig. 4E, because this fragment does not contain cGH sequences.

Dot blot hybridization of DNA from brain, liver, and muscle of four 14-day embryos infected before incubation showed that two of the four embryos contained vector-specific sequences in all three tissues. One embryo contained vector sequences in liver and muscle only, and one embryo was negative (Fig. 3).

Analysis of serum cGH. Circulating levels of cGH were determined by RIA of serum from thirty 15-day-old embryos infected with vector before incubation (Table 1). Concentrations of cGH in serum from 16 of 30 injected embryos (55%) were at least 10 times the level in uninjected control embryos, and they ranged from 18 to 254 ng/ml. All 35 control embryos contained less than 2 ng of detectable serum cGH per ml. Western blot analysis of cGH immunoprecipitated from serum of a number of these embryos is shown in Fig. 2, lanes 5 to 9. The amount of cGH present in serum from infected embryos is similar to the amount of cGH produced in vitro by infected culture cells.

Vector sequences in adult chickens. Southern blot analysis of DNA isolated from blood, brain, muscle, and testis of an adult chicken (no. 87725) which had been injected as an embryo with the ME111 vector is shown in Fig. 5. DNAs were digested with *Bam*HI and *Bgl*II before analysis. The four different probes used hybridized with the REV sequence present in the vector, HSV-1 *tk* sequences of the vector, REV structural gene sequences (absent from the vector), or endogenous cGH genes. All analyzed DNAs from bird 87725 contained the predicted DNA fragments of 0.74

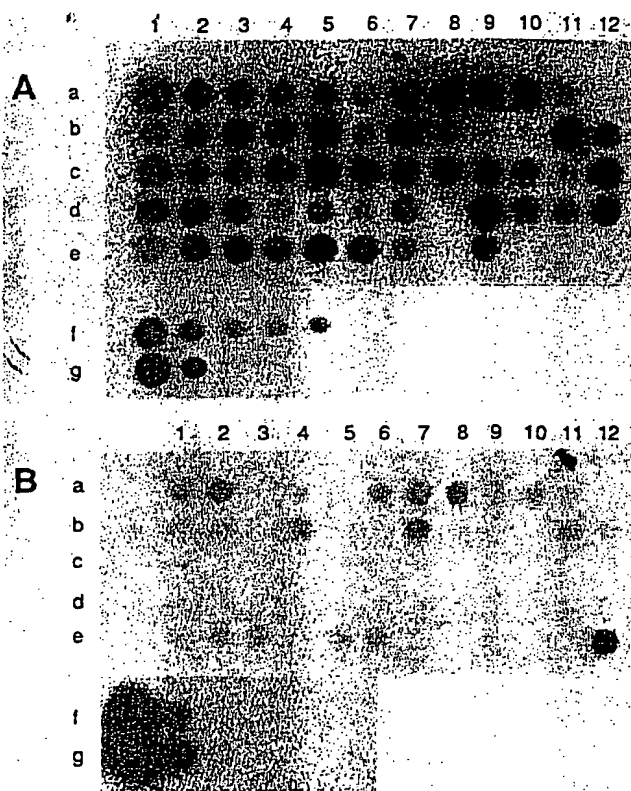


FIG. 3. Dot blot hybridization analysis of chicken embryo DNA. (A) Chicken embryo DNA hybridized with a cGH probe. Rows: a, b, and c1, total DNA from 7-day embryos injected with 20 μ l of vector C3-44; c2 to c12 and d1 to d7 and d9, DNA from uninjected control embryos; d10 to d12, e1 to e3, e4 to e6 and e7 to e9, DNA from brain, liver, and muscle of four 14-day embryos injected with vector SW272/cGH; f1 to f4 chicken blood DNA (~15 μ g) mixed with 1/10 dilutions of vector DNA starting with 1 ng in spot f1; f5, chicken blood DNA only; g1 to g4, yeast tRNA (5 μ g) mixed with the same amounts of vector DNA present in rows f1 to f4; g5, yeast tRNA only. (B) Same as in panel A, except filters were hybridized with a vector-specific probe (see Fig. 1). Row e12 is the same as row a1. Approximately 15 to 30 μ g of total embryo DNA was applied to each spot, using a 96-well blotting apparatus.

and 1.6 kb recognized by the REV vector probe and fragments of 1.2 and 1.7 kb recognized by the *tk* probe (Fig. 5A and C, respectively). DNA from blood and brain contained additional hybridizing fragments which probably include junctions between vector and cellular DNA at sites of integration (Fig. 5A, lanes 2 and 3). No REV-specific bands were observed in any of these tissue DNAs (Fig. 5B). Hybridization with cGH probe revealed endogenous fragments of ~2.7 and ~6.4 kb (Fig. 5D). D17 cells cocultivated with blood taken from bird 87725 at 4 weeks of age were reverse transcriptase-negative after 4 weeks of culture and did not produce detectable *tk* gene-transducing activity. Of 14 similarly derived birds, 2 were virus positive as determined by the same assay (17). Although the presence of low levels of replicating REV in birds like no. 87725 cannot be ruled out, these results are consistent with infection of embryonic stem cells with nonreplicating REV vectors.

Southern blot analysis of DNA from semen and blood of SW272/cGH-positive and control birds is shown in Fig. 6. Filters containing *Bam*HI-digested DNAs were hybridized

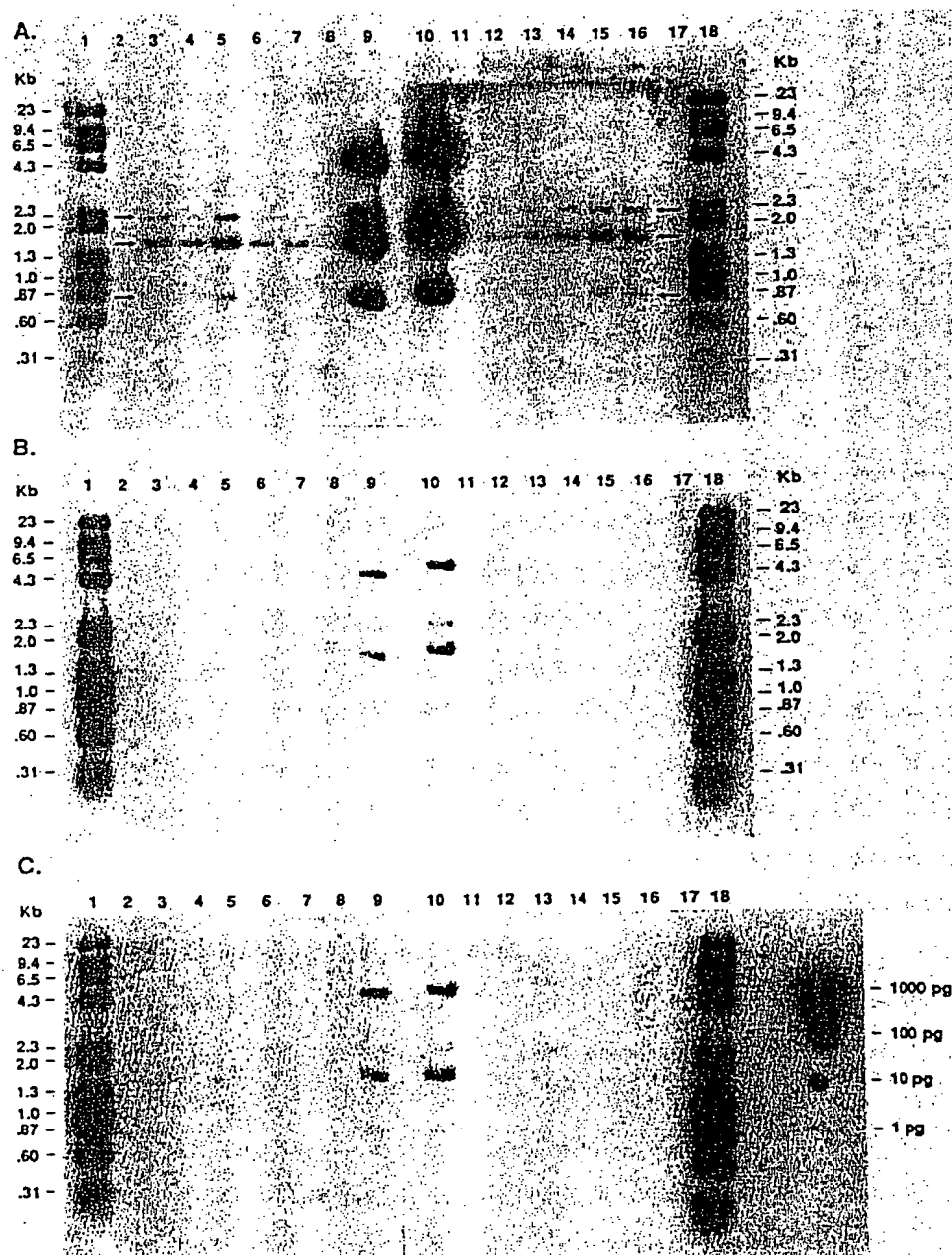


FIG. 4. Southern blot analysis of DNA from 7-day chicken embryos injected with 20 μ l of SW272/cGH vector before incubation. High-molecular-size DNA (15 μ g) was digested with *Bam*HI before analysis. The same filter was hybridized to three different probes: pSW272 probe (A), probe removed from panel A (B), virus-specific probe (C), probe removed from panel C (D), and cGH-specific probe (E). Probe hybridized to vector DNA in lanes 9 and 10 of panel A could not be completely removed. Sequences recognized by these probes are illustrated in Fig. 1. Lanes: 1 and 18, *Hind*III-digested lambda phage DNA, *Hae*III-digested ϕ X174 DNA, and *Bam*HI-digested uninjected chicken blood DNA; 2 and 11, DNA from uninjected embryos; 8 and 17, DNA from blood of uninjected chickens; 3 to 7 and 12 to 16, DNA from vector-injected embryos; 9 and 10, *Bam*HI-digested DNA of pSW272/cGH (1 ng) plus uninjected chicken blood DNA. *Bam*HI fragments internal to the proviral vector are marked with arrows in panel A. *Bam*HI fragments containing the endogenous cGH sequence are marked by asterisks in panel E. Dot blot on the right of panel C contains the indicated amounts of pSW253 containing the REV-A provirus (5). Sizes are shown in kilobase pairs (Kb).

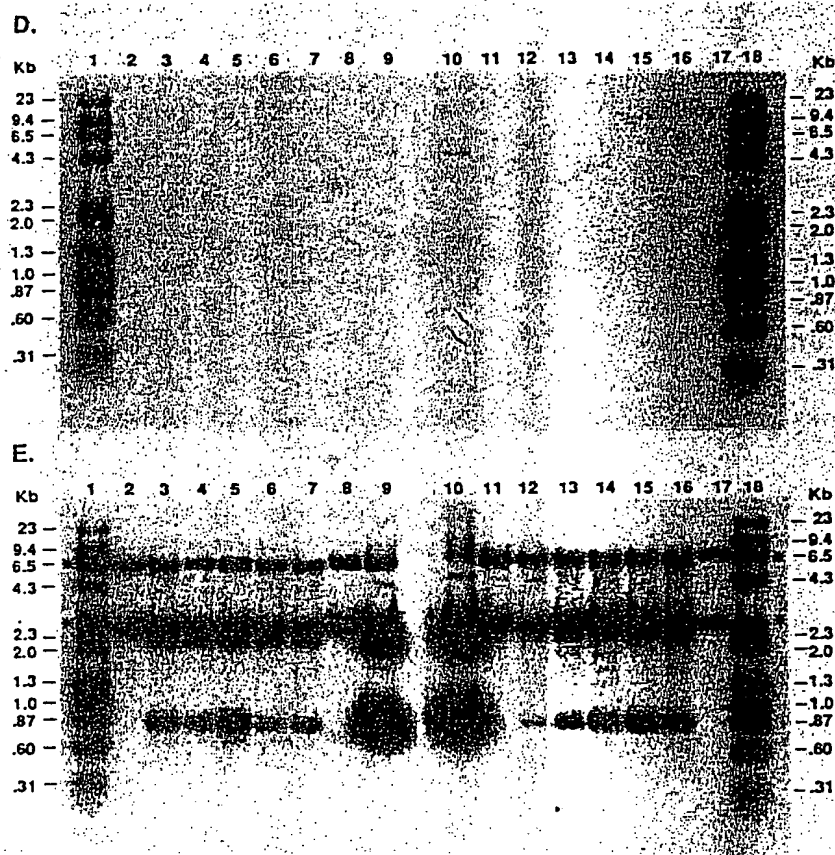


FIG. 4—Continued.

with radiolabeled DNA probes for the cGH coding sequence, 5' and 3' vector-specific sequences, or REV virus probe (see Fig. 1). Lane 1 of each panel contains a mixture of bacteriophage lambda and ϕ X174 DNAs digested with *Hind*III and *Hae*III, respectively, and *Bam*HI-digested control chicken blood DNA. Lane 9 in Fig. 6A to C contains *Bam*HI-digested control chicken blood DNA and plasmid DNA of pSW272/cGH. Internal *Bam*HI fragments of vector DNA are indicated by arrows. *Bam*HI fragments derived from the endogenous cGH gene are shown by the asterisks. Internal fragments derived from the complete REV provirus are marked by chevrons. Results obtained by hybridization with the cGH-specific probe are shown in Fig. 6A. *Bam*HI-digested DNAs from control semen and blood (lanes 3 and 6, respectively) contain endogenous fragments of ~2.7 and ~6.4 kb. In lanes 4 and 7, DNAs from the semen and blood, respectively, of the vector-positive male contain an additional 0.86-kb fragment which derives from the vector SW272/cGH and hybridizes to the cGH probe. Although visible in the original autoradiogram, the 2.3-kb *Bam*HI fragment derived from the vector is not well resolved from the strongly hybridizing 2.7-kb *Bam*HI fragment derived from the endogenous cGH gene. Blood DNA appears to contain much less of the 0.86-kb fragment than does semen DNA.

Panel B of Fig. 6 shows results obtained when a similar filter was hybridized with the vector probe. Lanes 4 and 7 show that semen and blood DNA from an infected male

contain *Bam*HI fragments of 0.86 and 1.6 kb. These fragments derive from the 5' and 3' ends of the integrated vector DNA, respectively. The additional 2.3-kb internal *Bam*HI fragment of vector DNA containing HSV-1 *tk* sequences does not hybridize to the vector probe used in Fig. 6B nor does *Bam*HI-digested DNA from semen and blood of uninfected control birds (lanes 3 and 6). No 1.4-kb fragment characteristic of replicating REV was observed. The patterns of semen and blood *Bam*HI DNA fragments hybridizing with these probes are similar to each other and are consistent with the pattern observed in *Bam*HI-digested DNA from infected embryos (Fig. 4).

Panel C of Fig. 6 shows results of hybridization with a virus-specific probe. Lanes 1 to 9 are as described for panels A and B. Lane 10 is blank. Lanes 11 and 12 contain *Bam*HI-digested plasmids pSW279 (39) and pSW253 (5), respectively. DNA in lane 11 has a 5' LTR derived from SNV (with a *Bam*HI site) but a 3' LTR derived from REV-A (without a *Bam*HI site). This provirus also lacks a 310-base-pair packaging sequence (E) located near the 5' end of the provirus. The expected fragments generated by *Bam*HI digestion of this DNA are present at ~1.1 (E⁻), ~1.8, ~2.2, and ~0.7 kb (visible at longer exposure times). Plasmid pSW253 in lane 12 contains the REV-A provirus and lacks the *Bam*HI site present in the SNV LTR. *Bam*HI digestion of this DNA generates the observed ~1.8- and ~2.2-kb fragments. The large fragment of ~9 kb in lane 12 contains 5' and 3' portions of the provirus and a portion of the *gag* gene

TABLE 1. cGH levels in chicken embryo serum^a

C3-44-injected embryos		Uninjected embryos	
Bird no.	Amt (ng/ml) of cGH	Bird no.	Amt (ng/ml) of cGH
1	51	31	<0.80
2	180	32	<0.80
3	100	33	<0.80
4	0.9	34	<0.80
5	41	35	<0.80
6	200	36	0.85
7	2.6	37	<0.80
8	80	38	<0.80
9	44	39	<0.80
10	106	40	<0.80
11	4.5	41	<0.80
12	18	42	1.2
13	8.6	43	1.0
14	1.1	44	<0.8
15	0.92	45	<0.8
16	2.2	46	<0.8
17	0.8	47	<0.8
18	254	48	<0.8
19	10.8	49	1.1
20	240	50	1.2
21	168	51	1.2
22	32	52	0.9
23	56	53	1.2
24	12	54	0.9
25	42	55	<0.8
26	0.86	56	<0.8
27	0.70	57	<1.1
28	3.4	58	<0.8
29	1.4	59	<0.8
30	0.76	60	<0.8
		61	<0.8
		62	<0.8
		63	<0.8
		64	<0.8
		65	<0.8

^a Embryos of unincubated eggs were injected with 10 μ l of medium from cultures of clone C3-44. After 15 days of incubation, serum from each embryo was assayed by RIA for cGH.

sequence. The 0.7-kb fragment is observed at longer exposure times. DNAs in lanes 3, 4, 6, 7, and 9 do not contain sequences detectable with probe derived from the structural genes of REV.

DISCUSSION

Early chicken embryo development. Fertilization and the first 24 h of chicken embryonic development occur in the oviduct and uterus, concomitant with the accretion of albumen and deposition of the eggshell. During this period, attempts at gene transfer into the embryo must allow for surgical removal after fertilization and either reintroduction to the oviduct or extensive artificial culture (25, 26). Both of these approaches are technically difficult. Alternatively, infection of the embryo just after ovaposition represents a strategy well suited to vector-mediated gene transfer. The embryo at this stage is composed of at least 10,000 cells arranged in a disk-shaped blastoderm, one to two cells thick and 2 to 3 mm in diameter (10, 20). The day-old blastoderm floats on the yolk above a fluid-filled subgerminal cavity.

Previous studies have provided a detailed description of early chicken embryo development (10, 20) and insights regarding the developmental potential of cells comprising the embryonic blastoderm of a freshly laid egg (9, 11, 12, 22).

Separated posterior and anterior portions of very young unincubated blastoderms appear totipotent, with similar ability to form embryos in vitro (10). The slightly older blastoderm exhibits cells of both upper epiblastic and lower hypoblastic layers. Separated from the lower layer of cells, the upper epiblastic layer retains its pluripotency, regenerates a new hypoblastic layer, and can subsequently form an early-stage embryo in vitro. When dissociated and grown in culture, epiblast cells form structures resembling embryoid bodies formed by murine teratocarcinomas (22). The hypoblastic layer, in contrast, survives but does not form embryonic structures (22).

All of the above observations suggest that successful infection of the early blastoderm with REV vectors might result in gene transfer into pluripotent embryonic stem cells. However, the REVs are primarily exogenous viruses in the chicken (41). Even though infected dams can transmit the virus vertically to their offspring by shedding virus into the egg (42), nucleic acid sequences closely related to REV are not endogenous to the chicken genome (Fig. 4C). The biology of virus-host interactions may preclude stable insertion of REV sequences into the chicken genome under natural conditions. Insertion of complete REV proviruses into the chicken genome could adversely affect viability, but even defective proviruses appear to be absent from the chickens analyzed in this study.

Infection of unincubated chicken embryo blastoderms. We have used replication-defective REV vectors ME111 and SW272/cGH to test the feasibility of retrovirus-mediated gene transfer in the chicken. The C3 helper cell line has been used to generate titers of about 10^4 infectious units per ml. The ME111 vector carries the Tn5 neomycin phosphotransferase gene and the HSV-1 tk gene and has been described previously (8). The vector SW272/cGH carries a cDNA sequence encoding the cGH mRNA and the HSV-1 tk gene (see Fig. 1). Clone C3-44 released about 10^4 TKTU/ml and expressed about 500 ng of cGH per ml of culture medium. Analysis by RIA (35) (data not shown) and Western blotting (3) (Fig. 2) showed that cGH released by C3-44 and transduced by SW272/cGH is similar to natural cGH.

Glass needles (40 to 60 μ m diameter) were used to deposit medium containing vector directly above and below the surface of the unincubated embryonic blastoderm. This method resulted in successful transduction of vector sequences into recipient embryos. Estimates of the amount of vector injected into the space beneath the blastoderm are based on the titer on BRLtk⁻ cells ($\sim 10^4$ TKTU/ml) and the observation that REV titer on chicken cells could be 10- to 100-fold higher (39). We estimate that between 10^3 and 10^4 TKTU were injected per embryo.

Vector DNA present in 7-day embryos. Dot blot analysis of 7-day embryo DNA shown in Fig. 3 indicated that about 50% of injected embryos contained detectable vector sequences. Three different radiolabeled probes were used in Southern blot analysis of high-molecular-mass DNA from 10 embryos to distinguish the REV structural genes, the SW272/cGH vector, and endogenous cGH sequences from each other (see Fig. 1). Since most infected embryonic cells are likely to have a single copy of vector, these blots indicate that a significant percentage of the embryonic cells may carry vector sequences 7 days after infection. This is most evident in comparisons of endogenous and vector-specific *Bam*HI fragments hybridizing to the cGH probe (Fig. 4E). The lack of *Bam*HI fragments specific for replicating REV (Fig. 4C) confirms that gene transfer is primarily the result of the replication-defective REV vector and not of contaminating helper virus (17). These results show that early embryonic

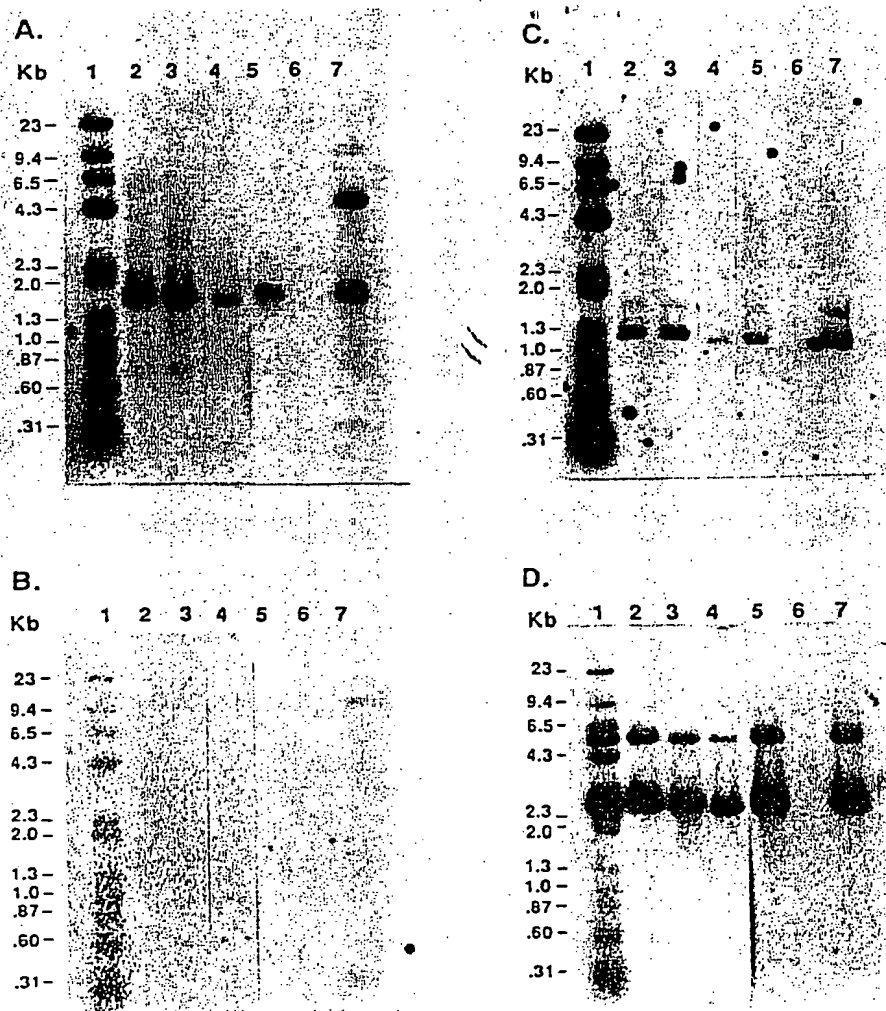


FIG. 5. Southern blot analysis of ~20 µg of *Bam*HI- and *Bgl*II-digested DNA from tissues of ME111-positive adult male 87725. (A through D) Replicate blots hybridized with radiolabeled vector probe (A), virus probe (B), *tk* probe (C), and cGH probe (D). Lanes: 1, *Hind*III-digested lambda phage DNA; *Hae*III-digested ϕ X174 DNA and *Bam*HI- and *Bgl*II-digested negative control chicken blood DNA; 2, blood DNA; 3, brain DNA; 4, muscle DNA; 5, testis DNA; 6, blank; 7, *Bam*HI- and *Bgl*II-digested pME111 (50 µg) and negative control chicken blood DNA. Sizes (in kilobases) are shown at the left of each panel.

cells are susceptible to REV infection and that they persist during development, comprising a significant fraction of the 7-day embryo.

Expression of cGH in vector-injected embryos. Expression of the endogenous cGH gene occurs late during embryonic development. Caudal cells of the pituitary do not contain immunodetectable cGH until day 12 of embryonic development (19), whereas detectable plasma cGH does not appear until day 17 of incubation (15). Furthermore, response of the pituitary to the cGH secretagogue, thyrotrophin-releasing hormone, is not seen until hatching (7). The absence of endogenous REV and the restricted location and timing of endogenous cGH expression facilitate the distinctions between endogenous and vector-encoded genes and their products.

Expression of the cGH gene in vivo resulted in elevated serum cGH levels in about 50% of injected embryos when measured after 15 days of development (Table 1). Levels of serum cGH in 30 injected embryos varied from <1 ng/ml to 254 ng/ml, whereas none of the 35 uninjected controls had

serum cGH levels above 2 ng/ml. Immunoprecipitated serum cGH from infected embryos comigrated with purified recombinant cGH as shown by Western blot analysis (Fig. 2). The relative contribution of somatic tissues to circulating levels of cGH is not known. These results are consistent with infection of embryonic stem cells present in the blastoderm at the time of vector injection and expression of vector-encoded cGH.

Vector DNA in tissues of adult males. Southern blot analysis of DNA from an adult male injected as an embryo with ME111 demonstrated the presence of vector in blood, brain, muscle, and testes (Fig. 5). Analysis of semen DNA by Southern blotting confirmed the presence of integrated un-rearranged vector sequences in a low percentage of the sperm cells from a bird injected with SW272/cGH (Fig. 6). The pattern of *Bam*HI restriction fragments observed (0.86 and 1.6 kb) is consistent with that seen in Southern blot analysis of DNA from infected embryos. Probe containing HSV-1 *tk* sequences revealed the additional 2.3-kb *Bam*HI

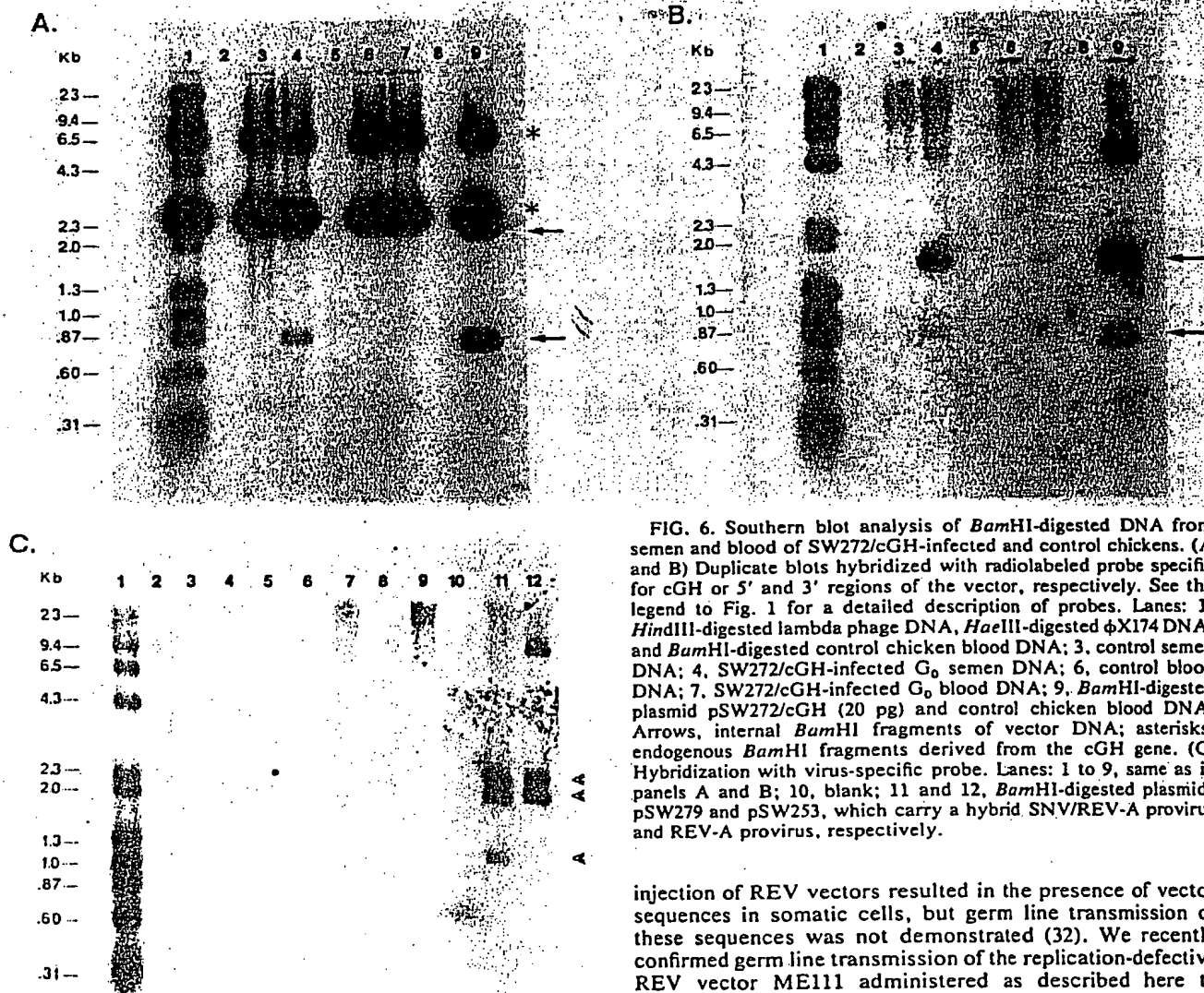


FIG. 6. Southern blot analysis of *Bam*HI-digested DNA from semen and blood of SW272/cGH-infected and control chickens. (A and B) Duplicate blots hybridized with radiolabeled probe specific for cGH or 5' and 3' regions of the vector, respectively. See the legend to Fig. 1 for a detailed description of probes. Lanes: 1, *Hind*III-digested lambda phage DNA, *Hae*III-digested ϕ X174 DNA, and *Bam*HI-digested control chicken blood DNA; 3, control semen DNA; 4, SW272/cGH-infected G_0 semen DNA; 6, control blood DNA; 7, SW272/cGH-infected G_0 blood DNA; 9, *Bam*HI-digested plasmid pSW272/cGH (20 pg) and control chicken blood DNA. Arrows, internal *Bam*HI fragments of vector DNA; asterisks, endogenous *Bam*HI fragments derived from the cGH gene. (C) Hybridization with virus-specific probe. Lanes: 1 to 9, same as in panels A and B; 10, blank; 11 and 12, *Bam*HI-digested plasmids pSW279 and pSW253, which carry a hybrid SNV/REV-A provirus and REV-A provirus, respectively.

vector DNA fragment (data not shown) also seen in Fig. 5A. Vector sequences present in semen are not caused by contaminating blood cells since blood contains lower levels of vector DNA per microgram of total DNA, as shown by Southern blotting. Furthermore, blood cells were not detected in vector-positive semen subjected to microscopic examination nor could vector sequences be detected in negative control semen containing 1% vector-positive blood from a different bird. We did not observe any consistent *Bam*HI fragments representing junctions between cellular and vector sequences, probably because of the polyclonal makeup and low overall percentage of cells carrying the vector. No 1.4-kb *Bam*HI DNA fragment characteristic of replicating SNV was observed (40) (see Fig. 1).

Previous work with replication-competent derivatives of Rous sarcoma virus showed that infection of unincubated chicken embryos resulted in germ line insertion of proviral DNA (28, 29). In contrast, the same approach using competent REV resulted in somatic infection but did not lead to germ line insertion of proviral DNA (29). Similarly, follicular

injection of REV vectors resulted in the presence of vector sequences in somatic cells, but germ line transmission of these sequences was not demonstrated (32). We recently confirmed germ line transmission of the replication-defective REV vector ME111 administered as described here to chicken embryos (1). Breeding studies are now in progress to determine whether semen from the chickens infected as embryos with defective REV vectors encoding cGH can accomplish germ line transmission of the vector DNA to progeny.

Conclusion. Replication-defective REV vectors can introduce new genetic information into the chicken by infecting somatic stem cells of the embryo. Susceptibility of these stem cells to infection by REV vectors provides another approach to the *in vivo* study of avian development (4) and vector-mediated gene expression. The possible applications of this technology are numerous.

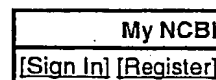
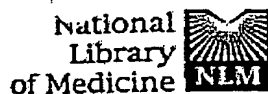
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LITERATURE CITED

1. Bosselman, R. A., R.-Y. Hsu, T. Boggs, S. Hu, J. Bruszewski, S. Ou, L. Kozar, F. Martin, C. Green, F. Jacobson, M. Nicolson, J.

- Schultz, K. Semon, W. Rishell, and R. G. Stewart. 1989. Germ-line transmission of exogenous genes in the chicken. *Science* 243:533-535.
2. Bosselman, R. A., R.-Y. Hsu, J. Bruszewski, S. Hu, F. Martin, and M. Nicolson. 1987. Replication-defective chimeric helper proviruses and factors affecting generation of competent virus: expression of Moloney murine leukemia virus structural genes via the metallothionein promoter. *Mol. Cell. Biol.* 7:1797-1806.
3. Burnette, W. N. 1981. Western blotting: electrophoretic transfer of proteins from sodium dodecyl sulphate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* 112: 195-203.
4. Cepko, C. 1988. Retrovirus vectors and their applications in neurobiology. *Neuron* 1:345-353.
5. Chen, I. S. Y., T. W. Mak, J. J. O'Rear, and H. M. Temin. 1981. Characterization of reticuloendotheliosis virus strain T DNA and isolation of a novel variant of reticuloendotheliosis virus strain T by molecular cloning. *J. Virol.* 40:800-811.
6. Cone, R. D., and R. Mulligan. 1984. High efficiency gene transfer into mammalian cells: generation of helper-free recombinant retrovirus with broad mammalian host range. *Proc. Natl. Acad. Sci. USA* 81:6349-6353.
7. Decuypere, E., and C. G. Scanes. 1983. Variation in the release of thyroxine, triiodothyronine and growth hormone in response to thyrotrophin releasing hormone during development of the domestic fowl. *Acta Endocrin.* 102:220-223.
8. Emerman, M., and H. M. Temin. 1984. Genes with promoters in retrovirus vectors can be independently suppressed by an epigenetic mechanism. *Cell* 39:459-467.
9. Eyal-Giladi, H. 1984. The gradual establishment of cell commitments during the early stages of chick development. *Cell Differ.* 14:245-255.
10. Eyal-Giladi, H., and S. Kochav. 1976. From cleavage to primitive streak formation: a complementary normal table and a new look at the first stages of the development of the chick. *Dev. Biol.* 49:321-337.
11. Eyal-Giladi, H., S. Kochav, and M. K. Menashi. 1976. On the origin of primordial germ cells in the chick embryo. *Differentiation* 6:13-16.
12. Eyal-Giladi, H., and N. T. Spratt, Jr. 1965. The embryo-forming potencies of the young chick blastoderm. *J. Embryol. Exp. Morph.* 13:267-273.
13. Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6-13.
14. Graham, F. L., and A. J. Van der Eb. 1973. A new technique for the assay of infectivity of human adenovirus in DNA. *J. Virol.* 52:456-467.
15. Harvey, S., T. F. Davison, and A. Chadwick. 1979. Ontogeny of growth hormone and prolactin secretion in the domestic fowl. *Gen. Comp. Endocrinol.* 39:270-273.
16. Hayward, W. S., B. G. Neel, and S. M. Astrin. 1981. ALV-induced lymphoid leukosis: activation of a cellular *onc* gene by promoter insertion. *Nature (London)* 290:475-480.
17. Hu, S., J. Bruszewski, M. Nicolson, J. Tseng, R.-Y. Hsu, and R. Bosselman. 1987. Generation of competent virus in the REV helper cell line C3. *Virology* 159:446-449.
18. Hughes, S. H., E. Kosik, A. M. Fadly, D. W. Salter, and L. B. Crittenden. 1986. Design of retroviral vectors for the insertion of new information into the avian germ line. *Poult. Sci.* 65: 1459-1467.
19. Jozsa, R., C. G. Scanes, S. Vigh, and B. Mess. 1979. Functional differentiation of the embryonic chicken pituitary gland studied by immunohistological approach. *Gen. Comp. Endocrinol.* 39: 158-163.
20. Kochav, S., M. Ginsburg, and H. Eyal-Giladi. 1980. From cleavage to primitive streak formation: a complementary normal table and a new look at the first stages of the development of the chick. *Dev. Biol.* 79:296-308.
21. Miller, A. D., and C. Buttimore. 1986. Redesign of retrovirus packaging cell lines to avoid recombination leading to helper virus production. *Mol. Cell. Biol.* 6:2895-2902.
22. Mitrani, E., and H. Eyal-Giladi. 1982. Cells from early chick embryos in culture. *Differentiation* 21:56-61.
23. Moscovici, C., M. G. Moscovici, and H. Jimenez. 1977. Continuous tissue culture cell lines derived from chemically induced tumors of Japanese quail. *Cell* 11:95-103.
24. Noori-Daloii, M. R., R. A. Swift, H.-J. Kung, L. B. Crittenden, and R. L. Winter. 1981. Specific integration of REV proviruses in avian bursal lymphomas. *Nature (London)* 294:574-576.
25. Perry, M. M. 1987. A complete culture system for the chick embryo. *Nature (London)* 331:70-72.
26. Rowlett, K., and K. Simkiss. 1987. Explanted embryo culture—*in vitro* and *in ovo* techniques for domestic fowl. *Br. Poult. Sci.* 28:91-101.
27. Salter, D. W., and L. B. Crittenden. 1987. Chickens transgenic for a defective recombinant avian leukosis proviral insert express subgroup A envelope glycoprotein. *Poult. Sci.* 66:170.
28. Salter, D. W., E. J. Smith, S. H. Hughes, S. E. Wright, and L. B. Crittenden. 1987. Transgenic chickens: insertion of retroviral genes into the chicken germ line. *Virology* 157:236-240.
29. Salter, D. W., E. J. Smith, S. H. Hughes, S. E. Wright, A. M. Fadly, R. L. Witter, and L. B. Crittenden. 1986. Gene insertion into the chicken germ line by retroviruses. *Poult. Sci.* 65: 1445-1458.
30. Scanes, C. G., and T. J. Lauterio. 1984. Growth hormone: its physiology and control. *J. Exp. Zool.* 232:443-452.
31. Sevoian, M., R. N. Larose, and D. M. Chamberlain. 1964. Avian lymphomatosis. VI. A virus of unusual potency and pathogenicity. *Avian Dis.* 8:336-347.
32. Shuman, R. M., and R. N. Shoffner. 1986. Gene transfer by avian retroviruses. *Poult. Sci.* 65:1437-1444.
33. Sorge, J., and S. H. Hughes. 1982. Splicing of intervening sequences introduced into an infectious retroviral vector. *J. Mol. Appl. Genet.* 1:547-559.
34. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503-517.
35. Souza, L. M., T. C. Boone, D. Murdock, K. Langley, J. Wypych, D. Fenton, S. Johnson, P. H. Lai, R. Everett, R.-Y. Hsu, and R. Bosselman. 1984. Application of recombinant DNA technologies to studies on chicken growth hormone. *J. Exp. Zool.* 232: 465-473.
36. Stoker, A. W., and M. J. Bissell. 1988. Development of avian sarcoma and leukosis virus-based vector-packaging cell lines. *J. Virol.* 62:1008-1015.
37. Sugden, B., K. Marsh, and J. Yates. 1985. A vector that replicates as a plasmid and can be efficiently selected in B lymphocytes transformed by Epstein-Barr virus. *Mol. Cell. Biol.* 5:410-413.
38. Swift, R. A., C. Boerkoel, A. Ridgway, D. J. Fujita, J. B. Dodgson, and H.-J. Kung. 1987. B lymphoma induction by reticuloendotheliosis virus: characterization of a mutated chicken syncytial virus provirus involved in *c-myc* activation. *J. Virol.* 61:2084-2090.
39. Watanabe, S., and H. M. Temin. 1982. Encapsidation sequences for spleen necrosis virus, an avian retrovirus, are between the 5' long terminal repeat and the start of the *gag* gene. *Proc. Natl. Acad. Sci. USA* 79:5986-5990.
40. Watanabe, S., and H. M. Temin. 1983. Construction of a helper cell line for avian reticuloendotheliosis virus cloning vectors. *Mol. Cell. Biol.* 3:2241-2249.
41. Witter, R. L., and D. C. Johnson. 1985. Epidemiology of reticuloendotheliosis virus in broiler breeder flocks. *Avian Dis.* 29:1140-1154.
42. Witter, R. L., E. J. Smith, and L. B. Crittenden. 1980. Tolerance, viral shedding, and neoplasia in chickens infected with non-defective reticuloendotheliosis viruses. *Avian Dis.* 25: 374-394.



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☐ 1: Biotechnology (N Y). 1991 Sep;9(9):835-8.

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Transgenic production of a variant of human tissue-type plasminogen activator in goat milk: generation of transgenic goats and analysis of expression.

Ebert KM, Selgrath JP, DiTullio P, Denman J, Smith TE, Memon MA, Schindler JE, Monastersky GM, Vitale JA, Gordon K.

Tufts University School of Veterinary Medicine, North Grafton, MA 01536-1895.

We report the first successful production of transgenic goats that express a heterologous protein in their milk. The production of a glycosylation variant of human tPA (LAtPA--longer acting tissue plasminogen activator) from an expression vector containing the murine whey acid promoter (WAP) operatively linked to the cDNA of a modified version of human tPA was examined in transgenic dairy goats. Two transgenic goats were identified from 29 animals born. The first animal, a female, was mated and allowed to carry the pregnancy to term. Milk was obtained upon parturition and was shown to contain enzymatically active LAtPA at a concentration of 3 micrograms/ml.

PMID: 1367544 [PubMed - indexed for MEDLINE]

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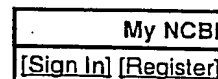
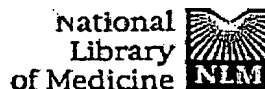
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1: Biotechnology (N Y). 1991 Sep;9(9):839-43.

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Transgenic expression of a variant of human tissue-type plasminogen activator in goat milk: purification and characterization of the recombinant enzyme.

Denman J, Hayes M, O'Day C, Edmunds T, Bartlett C, Hirani S, Ebert KM, Gordon K, McPherson JM.

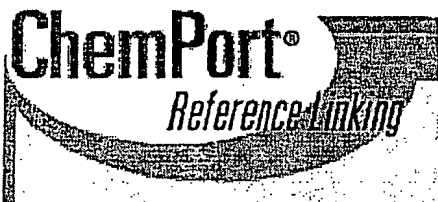
Genzyme Corporation, Framingham, MA 01701.

A glycosylation variant of human tissue-type plasminogen activator (tPA) designated longer-acting tissue-type plasminogen activator (LATPA) was extensively purified from the milk of a transgenic goat by a combination of acid fractionation, hydrophobic interaction chromatography and immunoaffinity chromatography. This scheme provided greater than 8,000-fold purification of the protein, a cumulative yield of 25% and purity greater than 98% as judged by SDS gel electrophoresis. SDS gel electrophoresis revealed that the transgenic enzyme was predominantly the "two chain" form of the protease. The specific activity of the purified transgenic protein, based on the average of the values obtained for three different preparations, was 610,000 U/mg as judged by amidolytic activity assay. This was approximately 84% of the value observed for the recombinant enzyme produced in mouse C127 cells. Analysis of the transgenic protein indicated that it had a significantly different carbohydrate composition from the recombinant enzyme produced in C127 cells. Molecular size analysis of the oligosaccharides from the transgenic and C127 cell-derived LATPA preparations confirmed their differences and showed that the mouse cell-derived preparation contained larger, complex-type N-linked oligosaccharide structures than the material produced in goat mammary tissue.

PMID: 1367545 [PubMed - indexed for MEDLINE]

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Gene transfer into sheep



Simons, J. Paul; Wilmut, Ian; Clark, A. John; Archibald, Alan L.; Bishop, John O.; Lathe, Richard

Bio/Technology (1988), 6(2), 179-83 CODEN: BTCHDA; ISSN: 0733-222X. English.

Six transgenic sheep were formed by microinjection of ONA into the pronuclei of single-cell eggs. Three DNA constructs were microinjected: (1) pMK, which contains the mouse metallothionein-1 (MT) promoter linked to the herpes simplex thymidine kinase gene nTK) (2) BLG-FIX, which contains the β -lactoglobulin gene (BLG) linked to cDNA sequences encoding for human blood-coagulation factor IX and (3) BLG- α 1AT, which contains gene BLG linked to cDNA sequences encoding human α 1-antitrypsin. The DNA in the transgenic sheep has not undergone rearrangement, as verified by hybridization assays. Hybridization intensities revealed the presence of single and multiple copies of constructs in the 6 lambs. Multiple copies had head-to-head and head-to-tail tandem arrangements. One of the offspring has the pMK construct, 4 of the offspring carry the BLG-FIX construct, and the last offspring carries the BLG- α 1AT construct. Offspring from these transgenic sheep also carry the transgenic genes.

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Expression of human anti-hemophilic factor IX in the milk of transgenic sheep



Clark, A. J.; Bessos, H.; Bishop, J. O.; Brown, P.; Harris, S.; Lathe, R.; McClenaghan, M.; Prowse, C.; Simons, J. P.; et al.



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Bio/Technology (1989), 7(5), 487-92 CODEN: BTCHDA; ISSN: 0733-222X. English.

Transgenic livestock may prove useful for the large scale production of valuable proteins. By targeting expression to the mammary gland these proteins could be harvested from milk. To this end, a hybrid gene was designed to direct the synthesis of human anti-hemophilic factor IX to the mammary gland, and introduced into sheep. Two transgenic ewes, each carrying about 10 copies of the foreign gene, have been analyzed for expression. Both animals express human factor IX RNA in the mammary gland and secrete the corresponding protein into their milk.

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